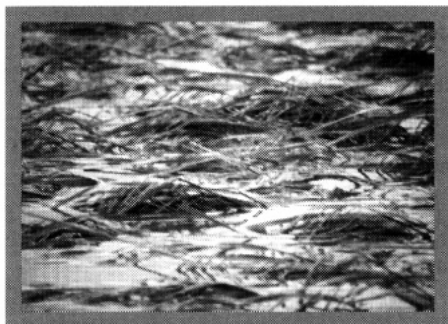


Biology and Management  
of the  
**Floodwater Ecosystem  
in Ricefields**

Pierre A. Roger



1996

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# Foreword

More than half the world's population depends on rice, which is grown on nearly 150 million hectares of land for a global production of more than 520 million tons. Wetlands — where rice grows in flooded fields during all or part of the cropping period — make up about 80% of world's rice area, accounting for 93% of all rice production.

It is estimated that by the year 2020 the annual demand for rice will exceed 760 million tons as world population swells to 8 billion, more than half of whom will be rice consumers. This production increase of almost 50% will have to be achieved on about the same amount of riceland than is cultivated today — or even less!

From a yield sustainability standpoint, traditional wetland rice cultivation has been extremely successful. Moderate but stable yields have been maintained for thousands of years without deterioration of the environment. But maintaining the sustainability of rice-producing environments in the face of increased demands will require new concepts and agricultural practices, which, in turn, will require increased knowledge of the ecology of the complex floodwater ecosystem.

Dr. Pierre Roger, currently director of research, French Institute for Scientific Research and Development Cooperation (ORSTOM), has made a dual contribution toward increasing knowledge of the ecology of floodwater ecosystems in ricefields. First, over a career of nearly 30 years (12 of these posted at IRRI), his research has contributed directly to our knowledge of soil microbiology in these environments. Second, through his extensive review of the literature in this book, which contains nearly 600 references published over the last 65 years, he has synthesized the major ecological and agronomic aspects of the biology of the floodwater environment.

In this book, the author focuses on management practices that maintain soil fertility, preserve or even improve the floodwater environment, and provide opportunities for diversifying sources of food and income beyond rice monoculture.

An appendix provides details on various methods for conducting ecological studies in ricefields and evaluates the different methods in relation to their suitability for different kinds of studies.

IRRI and ORSTOM have a long history of concern for sustainable development. We are proud that our collaboration has led to this compendium of knowledge about the ecology of wetlands, particularly as they relate to rice production.

GEORGE ROTHSCHILD  
Director General, IRRI

GÉRARD WINTER  
Director General, ORSTOM

# Abstract

The first part of the book describes the components of the ecosystem (floodwater, photosynthetic aquatic biomass, and fauna), their properties, and activities. Variations of the major physical and chemical properties of the floodwater (temperature, pH, O<sub>2</sub> concentration, N content) during the crop cycle are summarized. The section on photosynthetic aquatic biomass considers its components, composition, nutrient contribution, and effects on N dynamics, namely biological N<sub>2</sub> fixation, N immobilization, recycling, accumulation at the soil surface, supply to the rice crop, and N losses by NH<sub>3</sub> volatilization in relation to pH increase due to photosynthetic activity in floodwater. The section on fauna considers the nature of the components, their dynamics during the crop cycle, and their major activities: 1) grazing and nutrient recycling by primary consumers (zooplankton, snails), 2) nutrient translocation and recycling by aquatic oligochaete worms, and 3) the detrimental effects of the fauna on rice and as vectors of human diseases.

The second part considers the agronomic management of the floodwater ecosystem. First, it summarizes the effects of crop intensification on floodwater ecology and the possible related effects on soil fertility. Then it reviews the cultural practices that take advantage of the agronomic potential of the floodwater ecosystem by 1) promoting photodependent biological N<sub>2</sub> fixation by free-living and symbiotic cyanobacteria, 2) decreasing N losses due to NH<sub>3</sub> volatilization, and 3) producing additional sources of proteins through the introduction of edible animals, especially fish. Finally, it considers strategies to alleviate the effects of floodwater inhabitants that are detrimental as rice grazers or as vectors of human diseases.

Major methods for ecological studies in ricefields, including sampling strategies, are described in the appendix.

# Resumé

La première partie de l'ouvrage décrit les composants de l'écosystème (l'eau de submersion, la biomasse photosynthétique aquatique et la faune), leurs propriétés et leurs activités. Les variations des principales propriétés physiques et chimiques de l'eau de submersion (température, pH, concentration en O<sub>2</sub>, et en principaux éléments fertilisants) au cours du cycle cultural sont ensuite présentées.

Le chapitre sur la biomasse aquatique photosynthétique décrit ses composants principaux, leur composition chimique, leur dynamique au cours du cycle cultural et leur activités concernant le cycle de l'azote dans l'écosystème: la fixation biologique de N<sub>2</sub>, l'immobilisation de l'azote, son recyclage, son accumulation à l'interface entre le sol et l'eau, la contribution de la biomasse photosynthétique aquatique à la nutrition azotée du riz, et finalement les pertes d'azote par volatilisation de l'ammoniac en relation avec l'augmentation du pH de l'eau de submersion due à l'activité photosynthétique dans l'eau de la rizière.

Le chapitre sur la faune décrit ses composants, leur dynamique au cours du cycle cultural et leurs principales activités: 1) la consommation de la production primaire et le recyclage des éléments nutritifs par les consommateurs primaires (zooplancton et gastéropodes aquatiques), 2) la translocation et le recyclage des éléments nutritifs par les oligochaètes aquatiques et 3) les effets néfastes de la faune sur le riz et en tant que vecteur de certaines maladies humaines et animales.

La deuxième partie de l'ouvrage considère les différents aspects de la gestion agronomique de l'eau de la rizière. Après avoir présenté les effets des facteurs de l'intensification culturale sur l'écologie de la rizière et les conséquences possibles sur la fertilité des sols, les différentes méthodes agronomiques qui prennent en compte le potentiel agronomique de l'eau de la rizière en tant qu'écosystème sont présentées, à savoir: 1) la promotion de la fixation biologique photodépendante de l'azote par les cyanobactéries libres et symbiotiques (*Azolla*), 2) la diminution des pertes d'azote dues à la volatilisation de l'ammoniac et 3) la production de sources de protéines alimentaires par l'introduction dans la rizière d'animaux comestibles, en particulier des poissons. Le dernier chapitre fait le point sur les différentes

possibilités de contrôle des organismes vecteurs de maladies humaines ou animales qui se développent dans l'eau des rizières.

Les principales méthodes utilisables pour des études écologiques dans l'eau des rizières ainsi que les stratégies d'échantillonnage adaptées à cet écosystème sont présentées en annexe.

## About the author

Pierre A. Roger is Director of Research, French Institute for Scientific Research and Development Cooperation, Marseille, France. Before taking up his current post in 1992, he had been posted by ORSTOM as soil microbiologist at CNRS, Nancy, France (1967-69); ORSTOM, Senegal (1970-78); and at IRRI, Philippines (1979-91). His major areas of interest are soil microbiology, cyanobacteria, biological N<sub>2</sub> fixation, with particular emphasis on ricefield microbiology and ecology. Most recently, he has focused attention on the microbiological aspects of methane emission from ricefields. Roger is also the coauthor of *Blue-green algae and rice*, published by IRRI in 1980.

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# 1 Introduction

More than half of the world's population depends upon rice, which occupied 148 million ha of land in 1991 for a global production of 519 million t. Wetlands, where rice grows in flooded fields during all or part of the cropping period, make up about 87% of total riceland.

In terms of yield sustainability, traditional wetland rice cultivation has been extremely successful. Moderate but stable yields have been maintained for thousands of years without deterioration of the environment (Bray 1986). That is because flooding favors soil fertility and rice production by 1) bringing soil pH to near neutral, 2) increasing availability of nutrients, especially P and Fe, 3) retarding soil organic matter (OM) decomposition and thus, maintaining soil N fertility, 4) favoring  $N_2$  fixation, 5) suppressing outbreaks of soil-borne diseases, 6) supplying nutrients from irrigation water, 7) depressing weed growth, especially those of  $C_4$  type, and 8) preventing water percolation and soil erosion (Watanabe et al 1988a).

Flooding is especially important for maintaining soil N fertility when rice is grown year after year without added N fertilizer. In such conditions, biologically fixed N is considered responsible for maintaining crop yield. Its contribution, estimated from N balance studies, is 15-50 kg N/crop (Koyama and App 1979). De (1936) attributed much of the natural fertility of wetland ricefields to cyanobacteria (blue-green algae), the major indigenous  $N_2$ -fixing agent in ricefield floodwater.

An additional 300 million t of rice will be needed in 2020 to fill the demand of a fast-growing human population. That requires a 65% production increase within 30 yr without much expansion of actual cultivated area (IRRI 1989). But increased rice production should not be at the expense of future generations, nor should it ignore the concept of sustainability. It should be achieved through managing rice-producing environments to 1) satisfy changing human needs and maintain production over time in the face of ecological difficulties and social and economic pressure, 2) maintain or enhance the quality of the environment, and 3) conserve or enhance natural resources. Besides maintaining growth in productive agricultural systems and promoting growth in less productive systems, the major issues are 1) managing pests and nutrients in ways that reduce agrochemical use, 2) preserving the natural resource base, and 3) protecting the genetic base for agriculture.

The development of new concepts and agricultural practices to maintain the sustainability of the rice-producing environments will require an increased knowledge of the ecology of this complex freshwater ecosystem.

This book reviews some major ecological and agronomic aspects of the biology of the floodwater environment of tropical, shallow, wetland ricefields. The first part deals with the components of the ecosystem (floodwater, rice, photosynthetic aquatic biomass, and fauna), their properties and activities. The second part deals with agronomic management. It reviews the effects of crop intensification on floodwater ecology and the cultural practices that take advantage of the agronomic potential of the floodwater ecosystem.

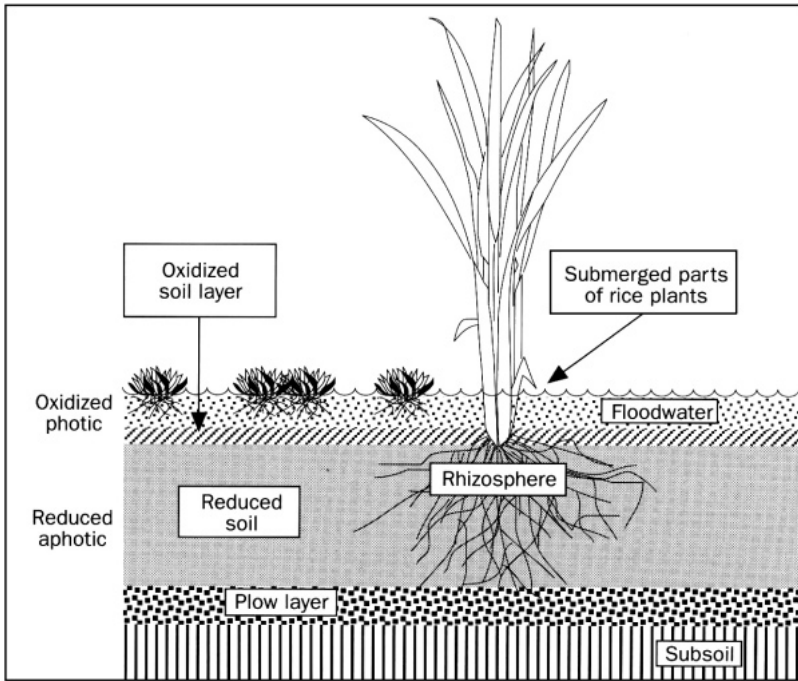
## **2 Major characteristics of the wetland ricefield ecosystem**

The flooded ricefield is usually a temporary aquatic environment subject to large variations of insolation, temperature, pH, O<sub>2</sub> concentration, and nutrient status. Cultivation restrains the secondary development of the ecosystem and prevents it from reverting to a marshland community (Watanabe and Roger 1985). Also, plowing, transplanting, and weeding disrupt community stability. Therefore, a specialized ricefield fauna and community structure might be expected to result. Soil chemistry probably influences the distribution of aquatic flora and fauna, so that associations characteristic of various soil types should be evident. But qualitative and quantitative observations are too scarce to permit comparisons.

The ricefield ecosystem is artificial and temporary, which makes it a less attractive environment to study. Frequent disturbances and agrochemical use interrupt classical ecological observations of community structure and energetics, nutrient cycling, and population succession (Grant et al 1986). As a result, ecological studies on the submerged soils of lowland ricefields are scarce as compared with those on freshwater lakes and rivers. Moroni (1961) conducted a pioneer ecological study in Italian ricefields. Heckman (1979) extensively studied a tropical ricefield in Thailand. Extensive environmental and ecological data on a deepwater ricefield in Bangladesh is also available (ODA 1984, Whitton et al 1988a, b).

Major environmental characteristics of the wetland ricefield ecosystem are determined by flooding, the presence of rice plants, and agricultural practices. Flooding creates anaerobic conditions a few millimeters beneath the soil surface. This leads to the differentiation of six major environments differing by their physical, chemical, and trophic properties: 1) floodwater, 2) surface-oxidized soil, 3) reduced soil, 4) plow pan, 5) subsoil, and 6) rice plants (submerged parts and rhizosphere) (Fig. 2.1).

Although those environments can be macroscopically differentiated, they are more or less continuous. In particular, continuous exchanges take place between floodwater and oxidized soil (Watanabe and Furusaka 1980). Macroenvironments might also be heterogeneous in their oxidation-reduction status at the microsite level because the activity of soil fauna creates microaerophilic sites in the reduced layer, while organic matter (OM) debris



2.1. Major environments of the wetland ricefield ecosystem.

and aggregates might provide anaerobic microsites in the oxidized soil layer and the water.

*The floodwater* is a photic aerobic environment where aquatic communities of chemosynthetic and photosynthetic producers (bacteria, algae, and aquatic weeds), invertebrate and vertebrate primary consumers (grazers), and secondary consumers (carnivorous insects and fish) provide OM to the soil and recycle nutrients. Because of the continuous exchanges taking place between floodwater and oxidized soil, Watanabe and Furusaka (1980) considered both environments as a continuum.

*The surface-oxidized soil layer* is a photic aerobic environment with a positive redox potential, a few millimeters thick, where  $\text{NO}_3^-$ ,  $\text{Fe}^{+3}$ ,  $\text{SO}_4^{-2}$ , and  $\text{CO}_2$  are stable and where algae and aerobic bacteria predominate. The depth of the oxidized layer is usually between 2 and 20 mm and depends upon the concentration of  $\text{O}_2$  dissolved in the floodwater, the reducing capacity of the soil, and the activities of the benthos and soil fauna. After land preparation, algae develop at the soil surface and support grazing populations. Later in the crop cycle, OM accumulates at the soil surface and supports populations of benthic filters and deposit feeders such as rotifers and molluscs. The activities of benthic invertebrates affect nutrient recycling either directly by excre-

tion or indirectly by release of native minerals through soil perturbation. The surface-oxidized zone is microbiologically very active.

*The reduced soil layer* is a nonphotic anaerobic environment where Eh is predominantly negative; reduction processes predominate;  $\text{NH}_4^+\text{-N}$ , sulphide, organic acids, and  $\text{CH}_4$  are produced; and where microbial activity is concentrated in soil aggregates containing organic debris. Decomposing OM in the reduced layer sustains populations of aquatic oligochaete worms and chironomid larvae. Animals inhabiting this zone frequently contain hemoglobin or possess air sacs as adaptations to low  $\text{O}_2$  concentrations.

*The plow pan* exhibits a lower permeability and a higher bulk density and mechanical strength than other soil layers. It reduces water and nutrient losses caused by leaching and percolation.

*The subsoil* below the plow pan is aerobic in well-drained soils and anaerobic in poorly drained soils. It is microbiologically active in its upper layer and its role in providing nutrients to rice, especially N, should not be underestimated (Ventura and Watanabe 1984).

*The rice plant* primarily affects the floodwater and surface soil by its shading effect, which increases as the rice canopy enlarges. The ensuing change in light intensity affects the growth of photodependent organisms. Rice also indirectly affects the floodwater and soil communities by lowering the temperature and the  $\text{CO}_2$  concentration under the canopy. Reduction of solar radiation coupled with low  $\text{CO}_2$  levels on calm days will influence growth rates, succession, and perhaps distribution of autotrophic organisms. Rice plants act as a substrate for epiphytic growths (Roger et al 1981) and provide mechanical support for many animal species. Pulmonate molluscs may escape high floodwater temperatures by resting on the stems at the air/floodwater interface.

*The rice rhizosphere* is a nonphotic environment where redox conditions are determined by the balance of the oxidizing and reducing capacities of rice roots, and where the production of C compounds by roots provides energy sources for microbial growth. The ability of the rice plant to transport  $\text{O}_2$  from the stem to the root and the diffusion of this  $\text{O}_2$  into the adjacent soil layer lead to the differentiation of an oxidized/reduced interface. Because rice roots can occupy a large volume, a significant fraction of the planted soil can be aerobic and the soil solution can maintain a high redox potential.

Major activities in the rhizosphere include 1) associative heterotrophic biological  $\text{N}_2$  fixation, 2) nitrification-denitrification, and 3) sulfate reduction (Watanabe and Furasaka 1980).



# 3 Floodwater management and properties

## 3.1. Water management in wetland rice cultivation

Adequate water management of wetland ricefields should consider the physiological requirements of the plant. As summarized by De Datta (1981), a low water level should be maintained at the early stages of the crop because 1) during the seedling stage, the development of radicles is reduced by the lack of  $O_2$  if seeds are submerged; and 2) during the early vegetative growth phase, a shallow water depth improves tillering and promotes firm anchorage of the plant in the soil. An adequate water level should be maintained during reproductive growth because rice is sensitive to drought and consumes a large quantity of water during this phase. During the ripening phase, rice needs little water, and fields are usually drained 10 d before harvest to facilitate mechanical harvest.

Depending on evapotranspiration, percolation and seepage, and the quantity of water used for land preparation and management, between 900 and 2,500 mm of water can be used per cropping season. Average consumption in irrigated ricefields at 43 sites in 7 rice-producing countries was 1,240 mm/crop (Kung 1971). An average rice production of 4 t/ha requires about 3 m<sup>3</sup> of water for each kilogram of grain produced.

According to De Datta (1981), continuous flooding with 5-7.5 cm of water is best for optimum grain yield, nutrient supply, and weed control with water depth and herbicide. However, about half the world rice area lacks water control, and in irrigated areas different methods of water management have been adopted.

In irrigated rice ( $80 \times 10^6$  ha, 53% of the world rice area), Khush (1984) defined the major types of water management as:

- continuous static flooding, with shallow (2.5-7.5 cm), medium (7.5-15 cm), or deep water (>15 cm) depth,
- continuous flowing irrigation, and
- rotational irrigation where the required amount of water is applied at regular intervals and the field may often be without standing water between irrigations, but the soil does not dry enough for stress to develop (De Datta 1981).

In about half the rainfed lowland rice area (25% of the world rice area), rainfall and water control are adequate to avoid droughts or submergences detrimental to rice. The other half includes shallow areas, which can be drought- and/or submergence-prone ( $10-12 \times 10^6$  ha), and rainfed medium-deep, waterlogged areas ( $5-6 \times 10^6$  ha).

There are about  $9 \times 10^6$  ha of low-lying ricelands on river deltas where 0.50 to more than 3 m water accumulates during the rainy season. Ricefields near sea coasts and estuaries are directly or indirectly influenced by tides.

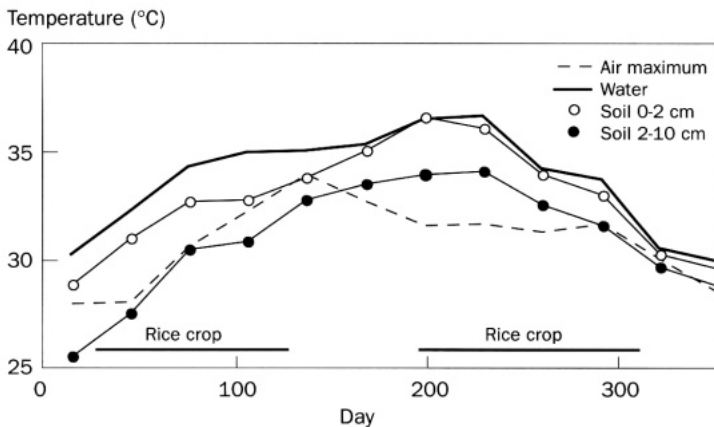
Because of water management, or the lack of it, and environmental conditions, water depth in ricefields may range from 0 to more than 3 m and its dynamics exhibit a wide range of patterns.

### 3.2. Physical and chemical properties of the floodwater

Physical and chemical properties of ricefield floodwater are characterized by variations that are often very marked both during the day and the crop cycle.

#### 3.2.1. Temperature

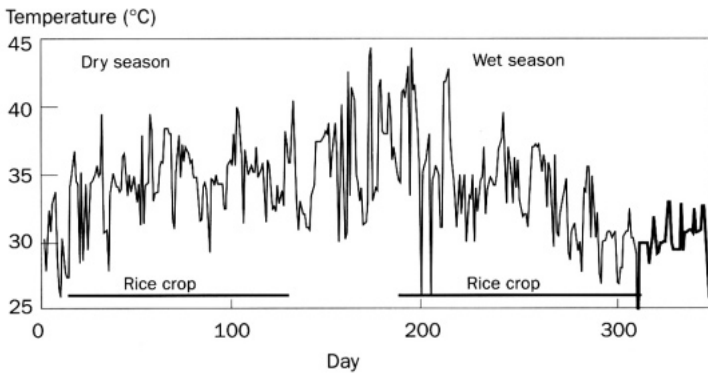
In flooded conditions, soil and water temperature depend upon 1) meteorological conditions (solar radiation, wind, air temperature, rainfall), 2) density of the rice canopy and aquatic plants, and 3) water depth and its dynamics. The changing properties of soil and floodwater (i.e., changes in reflectivity, heat capacity, thermal conductivity, muddiness and temperature of the incoming water, etc.) interact with these external influences. Floodwater transmits short-wave radiations to the soil while reducing the upward escape of emitted long-wave radiations. Thus a greenhouse effect is produced, heating



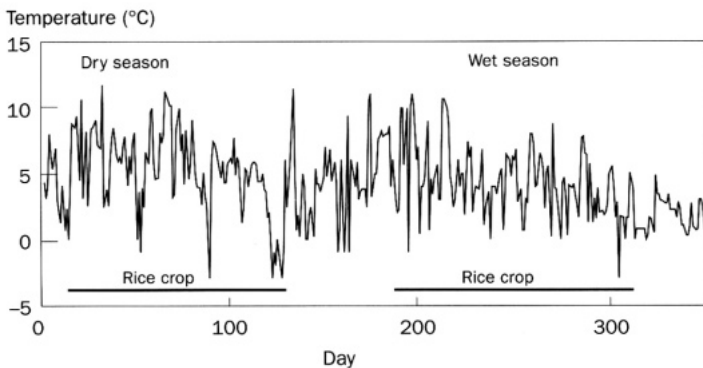
3.1. Average monthly values of maximum air temperature and of temperature in the floodwater, upper (0-2 cm) soil, and lower (2-10 cm) soil at 1400 h, IRRI farm, 1987.

floodwater and soil (Neue 1989). Therefore, highest temperatures in the ricefield ecosystem usually occur in the floodwater and at the soil surface (Fig. 3.1). The temperature of flooded rice soils may range from 15 to 40°C (Roger and Kurihara 1988). Maximum temperature, measured at the soil/floodwater interface during midafternoon, may often reach 36-40°C (Grant et al 1986) and exceed 40°C at the beginning of the crop cycle (Fig. 3.2).

Data collected by Heckman (1979) in Thailand show that floodwater temperature is positively correlated with air temperature, but may exhibit a higher maximum and usually exhibits narrower daily changes than those of air. Diel variations of floodwater temperature are moderated 1) by the high heat capacity of the water, and 2) by evaporation, which consumes energy from the water but not directly from the soil. Records in the Philippines showed daily changes ranging from 0 to 10 °C, and averaging 5 °C; daily variations decreased when the density of the rice canopy increased (Fig. 3.3). Maximum



**3.2.** Floodwater temperature at 1400 h daily, IRRI farm, 1987.



**3.3.** Diurnal variations (1400-0900 h) of floodwater temperature, IRRI farm, 1987.

diurnal change recorded in ricefields of New South Wales (Australia) was 16.1°C (Noble and Happey-Wood 1987).

De Datta (1981) summarized trends in water temperature fluctuations according to water management in irrigated rice as follows.

- Under continuous static shallow to medium flooding (2.5-7.5 cm), the fluctuation of the daytime water temperature is moderate, the range is less than for the fluctuations of air temperature.
- Under continuous static deep (>15 cm) flooding, the fluctuation of the daytime water temperature is low and the mean water temperature is generally about the same as the mean air temperature.
- Under continuous flowing irrigation, water temperature tends to be lower than the mean air temperature in the hot areas and warmer in the cold areas. The fluctuation of the daytime water temperature is usually low.
- Under rotational irrigation, temperature varies with the water level. Immediately after irrigation, conditions approximate those in the medium static flooding conditions. The fluctuation of the daytime water temperature increases as the water level decreases and remains high until the next irrigation.

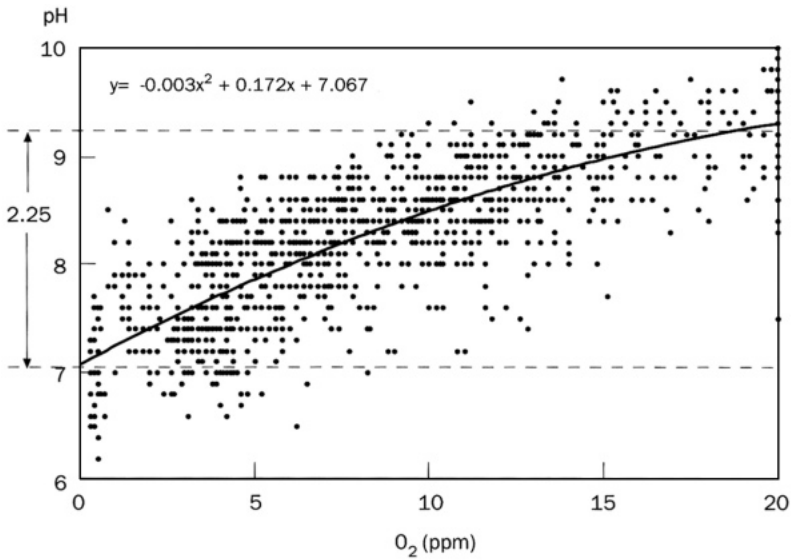
In unplanted ricefields with muddy and shallow water, the temperature may rise above 40 °C. When water is drained for transplanting or seeding, soil temperature may reach 50 °C in the top centimeter because of increased heat absorption and reduced heat capacity.

### 3.2.2. pH and O<sub>2</sub> Concentration

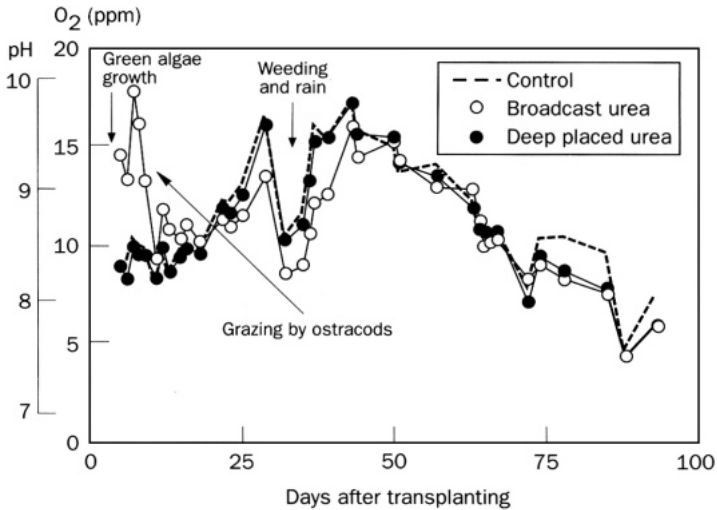
The concentration of O<sub>2</sub> in floodwater results from an equilibrium between production by the photosynthetic activity of the aquatic biomass, diffusion between air and water, and consumption by respiration and oxidation processes. As the partial pressures of CO<sub>2</sub> and O<sub>2</sub> are inversely proportional, pH and O<sub>2</sub> concentration in the floodwater are positively correlated (Fig. 3.4).

Curves of daily variations of O<sub>2</sub> concentration for rainfed (Heckman 1979) and irrigated fields (Saito and Watanabe 1978) show that, in the presence of submerged weeds, dissolved O<sub>2</sub> varies from supersaturation during the day to almost anoxic conditions at night.

Curves of daily variations of pH are available from studies of N losses by NH<sub>3</sub> volatilization following application of N fertilizer in the floodwater (Vlek and Crasswell 1979, Fillery et al 1984). They show that diurnal pH changes from a normally neutral floodwater to pH 9.5 are not uncommon at times of algal blooms. Figure 3.5 presents variations of pH and O<sub>2</sub> during a crop cycle. Both values increase with the development of the photosynthetic biomass till 30-40 d after transplanting (DT). Then, the increasing density of the rice canopy decreases the photosynthetic activity, and thus pH and O<sub>2</sub> concentration, till the end of the crop cycle.



3.4. Correlation between  $O_2$  concentration of the floodwater and pH in five flooded soils (P. A. Roger and P. M. Reddy, IRRI 1986, unpubl.).

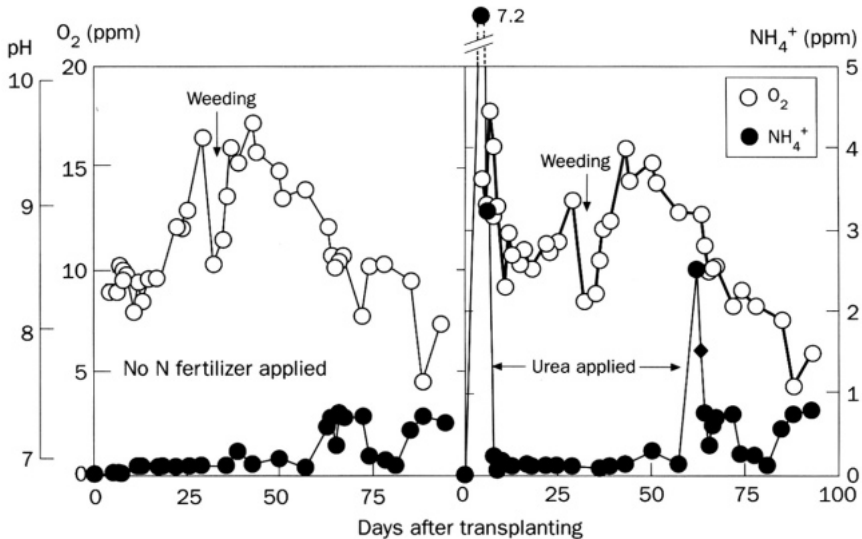


3.5. Dynamics of  $O_2$  concentration and pH of the floodwater of experimental plots at 1300 h according to N fertilizer status. Each value is the average of 4 replicated measurements in 20 plots. Measured  $O_2$  values are presented; pH scale is drawn from the regression curve between pH and  $O_2$  (Roger et al 1991a).

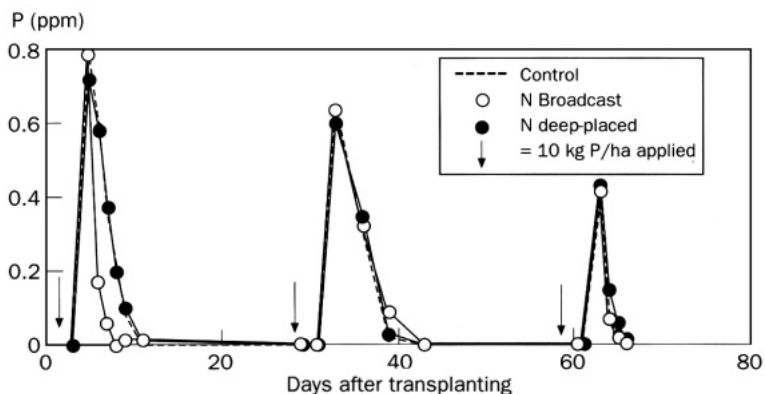
Concentration of  $O_2$  in the floodwater may range from a few to 20 ppm, while pH may exhibit variations of almost three units. Largest daily variations occur at the beginning of the crop cycle when explosive blooms of microalgae develop after N fertilizer is broadcast in the floodwater.

### 3.2.3. Nutrient concentrations and dynamics

The chemical composition of the floodwater depends on that of the irrigation water and the soil. Marked changes occur in response to dilution by rain, dispersion of the surface soil by cultivation practices and biological activities, and fertilizer application. Release of nutrients into the floodwater after land preparation, particularly following dry fallowing, is rapid for mineralized N (Shiga and Ventura 1976). It probably accounts for the initial algal bloom frequently seen after puddling (Kurasawa 1956, Saito and Watanabe 1978). Peaks of N and P usually decrease within 6-10 d following fertilizer application (De Datta et al 1983). When  $(NH_4)_2SO_4$  is broadcast,  $NH_4^+$  concentration in the floodwater may reach 40-50 ppm, although maximum  $NH_4^+$  concentrations 2-5 times lower were reported with urea (Fillery et al 1986, Bowmer and Muirhead 1987). Figure 3.6 shows that broadcasting urea at transplanting and panicle initiation increased  $NH_4^+$  concentration for about 6 d. Superphosphate application at intervals (Fig. 3.7) also increased P concentration in floodwater for about 1 wk.



3.6. Dynamics of pH and  $O_2$  and  $NH_4^+$  concentrations in the floodwater of experimental plots at 1300 h according to N fertilizer status. Each value is the average of four replicated measurements in five plots (P. A. Roger, S. Santiago-Ardales, and R. Remulla-Jimenez, IRRI 1988, unpubl.).



**3.7.** Dynamics of phosphorus concentrations in the floodwater of experimental plots according to N fertilizer status. Each value is the average of four replicated measurements in five plots (P. A. Roger, S. Santiago-Ardales, and R. Remulla-Jimenez, IRRI 1988, unpubl.).

The concentration of nutrients in floodwater is related to the activity of the photosynthetic aquatic biomass (PAB). Figure 3.6 shows a negative correlation between photosynthetic activity in the floodwater and  $\text{NH}_4^+$ -N concentration in plots where no N fertilizer was applied. The  $\text{NH}_4^+$ -N concentration resulting from the decomposition of the PAB remained lower than 1 ppm whereas 7.2 ppm was recorded after urea application (Fig. 3.6).

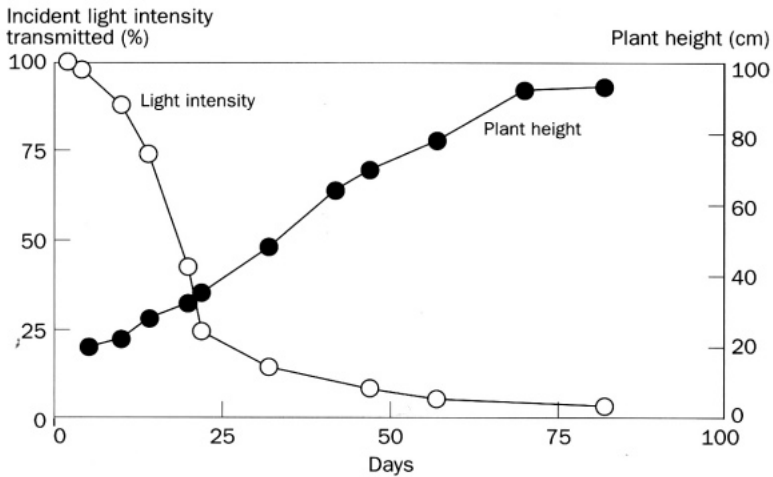
### 3.2.4. Light availability in floodwater

Depending upon season, latitude, cloud cover, and plant canopy, the light intensity reaching the floodwater varies from deficiency to high levels that may both limit or inhibit algal activity directly or indirectly.

In planted fields, the light-screening effect of the crop canopy appears to cause a rapid decrease of the light reaching the floodwater. Under transplanted rice, light reaching the water surface was reduced by 50% after 15 d, 85% after 30 d, and 95% after 60 d. The same measurements indicated that 30-cm plants suppressed 50% of the light and 60-cm plants cut off 90% (Kurasawa 1956) (Fig. 3.8).

Light penetration in the floodwater is impaired by floating macrophytes and microphytes, plankton, and floodwater turbidity. Turbidity results from cultural practices (land preparation, transplanting, and weeding), heavy rains, and the activity of benthic invertebrates, which stir up clay and silt particles.

As high temperatures are usually associated with high light intensities, it is important to interpret the relative effect of these two factors in ecological studies.



**3.8.** Relation between plant height and incident light intensity transmitted under the rice canopy (Kurasawa 1956).

### 3.3. Floodwater microflora

Reported counts of aerobic bacteria in the floodwater are about  $10^5$ - $10^6$ /ml (Rangaswami and Narayanaswami 1965, Suzuki 1967, Baldensperger 1981). A highly significant correlation between bacterial populations and the concentration of solids in the floodwater indicates that floodwater bacteria partly come from suspended surface soil particles (Watanabe and Furasaka 1980). This agrees with the observation by Baldensperger (1981) that variations of the number of bacteria in floodwater were not correlated with physicochemical parameters within the water, but were due to soil particle resuspended in the water by various mechanisms, including grazer activity. In his study, Baldensperger (1981) estimated the bacterial biomass to be 0.008 to 0.025 mg dry weight (dw)/liter of floodwater, which is equivalent to a few hundred g dw/ha.

# Photosynthetic aquatic biomass

After flooding, particularly when N and P fertilizers are applied, there is an upsurge in growth of photosynthetic organisms in the floodwater and at the soil surface. Most algae and aquatic macrophytes that develop in ricefields are also encountered in irrigation canals and surrounding water reservoirs, from where many of them originate.

The following chapter considers successively, the nature, the composition, the productivity, and the beneficial and detrimental roles of these organisms.

## 4.1. Components

The photosynthetic aquatic biomass (PAB) of primary producers that develop in ricefield floodwater is composed of 1) cyanobacteria or blue-green algae, 2) planktonic, filamentous, and macrophytic algae, and 3) vascular macrophytes (Table 4.1).

Cyanobacteria are photosynthetic prokaryotic microorganisms, which reproduce vegetatively only. Formerly they were classified with algae as blue-green algae. Morphologically, cyanobacteria can be classified into 1) unicellular and filamentous forms, and 2) scum-, mat- or macrocolony-forming groups. Physiologically, they can be classified into N<sub>2</sub>-fixing and non-N<sub>2</sub>-fixing forms.

Because cyanobacteria are basically primary producers in ricefield floodwater, they are considered here as part of the functional group of the *microalgae*, and not independently of eukaryotic algae as a taxonomical treatment would require.

Algae are primitive eukaryotic plants without true leaves or seeds. They reproduce vegetatively and through spores. Morphologically, three types can be distinguished:

- Phytoplanktons, which include microscopic single-celled and colonial forms.
- Filamentous algae such as *Cladophora* (cotton mat type), *Spirogyra* (slimy and green type), and *Hydrodictyon* (water net type), which frequently form scum.
- Higher algae such as *Chara* and *Nitella*, which resemble vascular plants, grow as anchored species, and possess stems and branches.

**Table 4.1. Components of the photosynthetic aquatic biomass in wetland ricefields.**

Group		Examples	
Cyanobacteria (prokaryotic algae)	Non-N <sub>2</sub> -fixing	Unicellular Filamentous	<i>Microcystis</i> <i>Oscillatoria</i> , LPP group <sup>a</sup>
	N <sub>2</sub> -fixing	Unicellular Filamentous	<i>Aphanothece</i> <i>Nostoc</i> , <i>Anabaena</i>
Microalgae (eukaryotic algae)	Unicellular		<i>Chlorella</i>
	Filamentous	Cotton mat type	<i>Cladophora</i>
		Slimy and green type	<i>Spirogyra</i> , <i>Mougeotia</i>
	Water net type	<i>Hydrodictyon</i>	
Macrophytic algae			<i>Chara</i> , <i>Nitella</i>
Macrophytes	Submerged		<i>Hydrilla</i>
	Floating		<i>Lemna</i> , <i>Pistia</i> , <i>Azolla</i>
	Immersed		<i>Typha</i> , <i>Eichhornia</i>

<sup>a</sup>LPP group = *Lyngbia*, *Plectonema*, *Phormidium* (Rippka et al 1979).

Algae and cyanobacteria, as phototrophic microorganisms, are restricted to the photic zone-floodwater and the upper 0.5 cm of soil. However, viable propagules occur up to a depth of 1 m (Kamat and Patel 1973). During day-time, vertical migration of phytoplankton and filamentous algae occurs in the water in relation to O<sub>2</sub> production by photosynthesis.

Aquatic macrophytes are usually divided into three groups:

- Submerged macrophytes rooted to the soil. They produce most of their vegetative growth beneath the surface. *Chara*, *Nitella*, and *Hydrilla* are common representatives of this group in ricefields.
- Surface (or floating) macrophytes, which have most of their leaves and flowers near the water surface. Both rooted and free-floating species occur in this group, characterized by special parenchymatous tissues for buoyancy. Genera frequently found in ricefields include *Pistia*, *Lemna*, *Azolla*, and *Nuphar*.
- Immersed or marginal macrophytes growing in shallow water or wet soils. This group contains most rooted plants considered as weeds.

Productivity of aquatic plants differs according to this classification. Ambasht and Ram (1976) studied the vertical distribution of dry matter (DM)

and chlorophyll in different aquatic communities and distinguished three types of productivity: 1) the upright triangle type, represented mainly by immersed plants (e.g., *Eleocharis*), whose photosynthetic biomass is concentrated just above the basal layer; 2) the inverted triangle type, represented by submerged plants (e.g., *Hydrilla* or *Najas*), whose photosynthetic biomass is greatest in the top layer of the water body; and, 3) the flag type, represented by floating species (e.g., *Nymphaea*), whose photosynthetic organs are concentrated on or above the water surface.

## 4.2. Composition

### 4.2.1. Phytoplankton

Milner (1953) pointed out the scarcity of information on the composition of freshwater phytoplankton, which is still true today. In addition, most data were collected on laboratory strains, which may have led to biased estimates. Laboratory strains are usually grown in optimized nutritional conditions and under light intensities much lower than those in natural environments. As an inverse correlation between light intensity and photopigment content is generally observed in photosynthetic organisms, one expects higher pigment content and lower ash content in laboratory-grown algae, which leads to a higher relative protein content.

Table 4.2 gives the composition of some freshwater eukaryotic microalgae. Data on *Chlorella* show how variable the composition of a species can be. But the range of variation in the composition of this genus may be mainly because no other plant has been subjected to such extensive experimentation on the effects of environmental conditions and chemical composition. Other species might show as much variation as *Chlorella* (Milner 1953).

The composition of cyanobacteria genera most frequently encountered in ricefields was studied on laboratory cultures, soil cultures, and natural Sam-

**Table 4.2. Composition of some eukaryotic freshwater algae (Roger and Watanabe 1984).**

Species	Ash (%dw) <sup>a</sup>	Proteins	Carbohydrates (% dw ash free)	Lipids	C:N
<i>Chlamydomonas</i> spp.	4.74	36.3	58.2	5.5	8.2
<i>Oikomonas termo</i>	5.08	33.5	45.8	20.7	9.7
<i>Synechococcus bacillaris</i> (A) <sup>b</sup>	6.50	62.3	25.8	11.9	5.3
<i>Synechococcus bacillaris</i> (6)	11.24	22.6	38.5	38.9	15.8
<i>Chlorella pyrenoidosa</i> (A)	3.45	58.0	37.5	4.5	5.3
<i>Chlorella pyrenoidosa</i> (6)	3.46	8.7	5.7	85.6	49.1
<i>Anabaenopsis</i> sp	9.35	45.5	45.6	8.9	6.8

<sup>a</sup> dw = dry weight. <sup>b</sup>A and B refer to two independent measurements. <sup>c</sup>Cyanobacteria for comparison.

ples by Roger et al (1986b) to assess their potential as biofertilizer. Data are summarized in Table 4.3.

Dry matter content ranged from 0.3 to 13.6% (average 3.8%). It was related to the nature of the strain, but large variabilities were also observed between species of the same genus. For example, values ranged from 1.0 to 8.5% for *Anabaena* and from 0.3 to 6.3% for *Nostoc*. *Fischerella* had the highest values (13%). Genera forming mucilaginous colonies (*Aphanothece*, *Nostoc*, *Gloeotrichia*) had the lowest values (0.3%). Such genera can develop impressive blooms, but the corresponding DM (1-2%) is low. Roger et al (1985b) recorded blooms of *Aphanothece* and *Nostoc* ranging from 7 to 33 t fresh weight (fw)/ha, but corresponding to 74 to 132 kg dry weight (dw)/ha and only 1.5-2.6 kg N/ha.

Ash content was 6-12% in laboratory cultures and 31-71% in field samples consisting mainly of mucilaginous strains. As with most aquatic plants, ash content of cyanobacteria is related to the quantity of soil in suspension in the floodwater. It is especially high in sheath-forming strains that adsorb clay and silt in their mucilage. A field bloom of mucilaginous *Aphanothece* and

**Table 4.3. Composition of laboratory cultures and field samples of cyanobacteria (Roger et al 1986b).**

Nature of the material		Composition <sup>a</sup>					
		Dry matter (%fw)	Ash (% dw)	N (% dw ash free)	C (% dw ash free)	C:N	P (% dw)
Mass cultures at exponential phase, n = 7	Mean	... <sup>b</sup>	7.4	8.02	45.1	5.6	0.59
	Max	...	11.8	9.24	49.3	6.3	0.75
	Min	...	5.6	6.90	43.5	4.8	0.40
Laboratory flask cultures < 4 wk old, n = 24	Mean	3.85	...	5.89	44.7	8.1	...
	Max	8.50	...	8.88	72.0	13.0	...
	Min	0.28	...	3.65	34.3	4.8	...
Laboratory flask cultures > 4 wk old, n = 24	Mean	3.56	...	4.64	...	...	...
	Max	13.64	...	8.60	...	...	...
	Min	0.30 ...	...	1.92	...	...	...
Artificial blooms produced on soil, 4 composite samples	Mean	4.02	26.7	6.26	42.8	7.1	0.31
	Max	6.41	37.2	7.43	45.4	10.0	0.39
	Min	0.94	15.0	4.00	40.0	5.3	0.14
Field samples, n = 11	Mean	...	52.1	4.81	39.9	8.5	0.12
	Max	...	71.3	5.97	45.1	11.6	0.18
	Min	...	30.7	3.82	36.6	6.6	0.05

<sup>a</sup> fw = fresh weight, dw = dry weight. <sup>b</sup> ... = not determined.

*Gloeotrichia* had an ash content of 59% after colonies were carefully washed (Roger et al 1986b).

Nitrogen content in ash-free material ranged from 1.9 to 11.8% (average 6%) of dw. It decreased in aging material. In laboratory cultures it averaged 8.0% at the exponential phase of growth, 5.9% in cultures < 4 wk old, and 4.6% in cultures > 4 wk old. Average N content in ash-free DM of material grown on soil was 6.8% in 2-wk-old material and 6.3% in 4-wk-old material. Field samples harvested after several weeks of growth contained 4.8% N.

Carbon content averaged 44% and exhibited low variability compared with other elements (CV = 7% for material grown on soil).

Phosphorus content was much lower in field samples (0.05-0.12%) than in laboratory cultures (average 0.6%). Data summarized by Healey (1982) suggest that P content in cyanobacteria growing in a P-sufficient medium is around 1 % or higher. According to these data, all field samples were deficient in P.

Pooled data showed a very large variability of the composition. For example, the ratio between higher and lower values recorded for N content was > 6. The composition was influenced at least as much by the growth conditions and the age of the strains as by their nature. This was shown by an average intrageneric variability (CV = 39%) slightly higher than the intergeneric variability (CV = 35%). Pooled data included those from laboratory cultures in which variability is, as a rule, wider than that observed in situ (Komarek 1971). The CVs were 31% for N and 20% for C in laboratory cultures, but 16% for N and 7% for C in field samples. However, variability in natural samples was still high.

Combining average and extreme values obtained for DM, ash, and N contents showed that N content in 1 t of fresh cyanobacteria averages 1.25 kg but may vary from 4 to 0.1 kg. Therefore, the data on algal biomass, expressed in kg fw/ha or dw/ha, give little information on its agronomic significance, which depends mainly on its N content.

#### **4.2.2. Freshwater macrophytes**

Because of increasing interest in the pollution problems in water bodies, more information is available on the composition of higher algae and aquatic weeds than on microalgae. Boyd has probably done the most comprehensive analyses of aquatic macrophytes, culminating in an extensive review (Boyd and Scarsbrook 1975) that tabulates data on temperate species from 35 papers. Little (1979) summarized papers on both tropical and temperate species and concluded that except for water the ingredients of aquatic plants are similar to those of dryland plants.

A high water content is the overwhelming characteristic of aquatic plants. Little and Henson (1967) presented results suggesting an average water content of 92%. By comparison, terrestrial forages contain 70-90% water.

A second characteristic of aquatic plants is a high ash content, which varies with site and season (Sculthorpe 1967). Sand, silt, and encrusted carbonates often account for much of the mineral content. Although silt is most frequently removed during analysis, in practice it represents part of the chemical composition of the harvest. Submerged macrophyte communities contain, on the average, 21.3% ash on a dw basis; floating communities average 11.5% (Sculthorpe 1967); upland plants usually contain less than 10%.

A third characteristic of aquatic plants is the large variability of composition (as in algae), which is influenced by the composition of the water in which they grow. Lawrence and Mixon (1970) have shown how aquatic plants growing in water containing ample quantities of N, P, and K will exploit the situation by *luxury consumption* of these elements, far more than the amount they need for healthy growth. The amount of mineral elements can be exceptionally high in aquatic plants grown in sewage or agricultural and industrial wastewater. An extensive example was the K uptake by *Alternanthera philoxeroides*: in one case, consumption was 20 times the content of plants grown in unfertilized pools (7.3% vs 0.36%). Table 4.4 shows the variability of the composition of water hyacinth. A compilation of data on the composition of some common aquatic weeds (Table 4.5) shows that they contain appreciable quantities of N, P, and K. On a DM basis, they have N and P contents similar to those of alfalfa, but a higher K content. The amounts of Mg, Na, S, Mn, Cu, and Zn in aquatic weeds growing in nature are generally similar to those in terrestrial plants. However, aquatic plants are often richer in Fe and Ca than forage plants (NAS 1976).

Table 4.6 compares the average composition of field samples of phytoplankton (cyanobacteria) and aquatic macrophytes. The average composition of aquatic macrophytes is about 8% DM, 2-3% N (dw basis), 0.2-0.3% P, and 2-3% K. Cyanobacteria have a lower DM content (averaging 4%) and a higher N content (3-5%). Data show that the components of PAB in

**Table 4.4. Variability of the composition of water hyacinth (Roger and Watanabe 1984).<sup>a</sup>**

Composition <sup>b</sup>	Data (no.)	Mean	Value		
			Lower	Higher	CV (%) <sup>c</sup>
Dry matter (% fw)	13	7.79	4.50	12.00	23.7
N (% DM)	9	1.86	1.03	3.70	42.9
P (% DM)	8	0.36	0.10	0.63	43.9
K (% DM)	7	3.35	1.81	4.40	28.8
Crude protein (% DM)	8	13.36	6.50	19.8	32.9
Ash (% DM)	6	13.40	11.90	25.6	28.3

<sup>a</sup>Data used for calculation were collected from the papers summarized by Little (1979). Data given on a fresh weight basis without indication of dry matter content of the plant were recalculated using a 7.79 % dry matter basis. <sup>b</sup>fw = fresh weight, DM = dry matter. <sup>c</sup>Variance expressed as percentage of the mean.

**Table 4.5. Composition of some aquatic weeds (Roger and Watanabe 1984).<sup>a</sup>**

Aquatic weed	Composition <sup>b</sup>				
	Dry matter (% fw)	Protein (%DM)	N (%DM)	P (%DM)	K (%DM)
<i>Chara vulgaris</i>	...	7.92	1.27 <sup>c</sup>	0.19	0.84
<i>Ceratophyllum</i> spp.	8.50	...	3.30	0.47	5.90
<i>Elodea canadensis</i> <sup>d</sup>	9.03	...	3.29	0.51	3.26
<i>Hydrilla</i> sp. <sup>d</sup>	8.00	17.1	2.70	0.28	2.90
<i>Lagarosiphon</i> spp. <sup>d</sup>	8.90	...	3.54	0.53	2.56
<i>Lemna minor</i>	...	17.86	2.87 <sup>c</sup>	0.17	1.20
<i>Myriophyllum</i> sp. <sup>d</sup>	...	...	2.81	0.43	1.75
<i>Nuphar variegatum</i>	...	15.70	2.52 <sup>c</sup>	0.23	1.62
<i>Pistia stratiotes</i>	5.90	13.2	2.1	0.30	3.50
<i>Potamogeton</i> spp. <sup>d</sup>	...	...	2.51	0.33	2.28
<i>Typha</i> spp. <sup>d</sup>	...	...	1.37	0.21	2.38
<i>Vallisneria</i> spp. <sup>d</sup>	...	13.5	2.14	0.20	5.70
<i>Eichhornia vaginalis</i> <sup>d</sup>	7.8	13.4	1.86	0.36	3.35
Average	...	...	2.48	0.32	2.86
Alfalfa hay <sup>e</sup>	15	...	2.7	0.26	1.77

<sup>a</sup>Data from Boyd (1969, 1970), Fish and Will (1966), Lancaster et al (1971), Lawrence and Mixon (1970), Lin et al (1975), and Riemer and Toth (1969), all cited in Little (1979). <sup>b</sup>fw = fresh weight, DM = dry matter, ... = not determined or not available. <sup>c</sup>Extrapolated from protein content (protein = 6.23 x N). <sup>d</sup>Average value. <sup>e</sup>For comparison.

**Table 4.6. Comparison of the composition of field samples of N<sub>2</sub>-fixing cyanobacteria and aquatic macrophytes (Adapted from Roger and Watanabe 1984 and Roger et al 1986b).**

Component <sup>a</sup>	Cyanobacteria		Aquatic macrophytes	
	Average	Range	Average	Range
Dry matter (% fw)	4.0	0.9-7.0	8.0	4.5-12.0
Ash (% dw)	45.0	27-71	20.0	12-50
N (% dw ash free)	5.0	3.8-7.4	2.1	1.3-2.9
C (% dw ash free)	40.0	37-47	... <sup>b</sup>	...
C:N	8.0	5-12	24.0	18-47
P (% dw ash free)	0.2	0.05-0.39	0.3	0.1-0.6

<sup>a</sup>DM = dry matter, fw = fresh weight, dw = dry weight. <sup>b</sup>... = not available.

ricefields usually have low DM content and high ash content (Roger and Watanabe 1984, Roger et al 1986b).

### 4.3. Standing crops and productivity

#### 4.3.1. Standing crops in ricefields

The development of PAB depends on the availability of nutrients and light. Largest biomasses are recorded in fallow plots and in fertilized fields when the rice canopy has not become too dense.

Probably because of technological difficulties in estimating algal abundance, quantitative evaluation of algal biomass in weight per unit area is scarce and deals mostly with cyanobacteria. Available data (Table 4.7) show that algae can develop a biomass of several tons fw/ha. The distribution of 400 records of the biomass of cyanobacteria in an experimental design of 65 plots that were subjected to a range of fertilizer treatments (Fig. 4.1) shows a me-

**Table 4.7. Biomass of algae and aquatic macrophytes in ricefields and freshwater bodies (Adapted from Roger 1987 and Roger and Watanabe 1984).**

Nature	Fresh wt (t/ha)	Dry wt (t/ha)	Site	Reference
<i>Ricefields</i>				
Cyanobacteria	7.5	0.37 <sup>a</sup>	China, ricefield	Academia Sinica 1958 <sup>c</sup>
Cyanobacteria	0.8 <sup>a</sup>	0.03	India, ricefield	Mahapatra et al 1971 <sup>c</sup>
Cyanobacteria	0-2.3	0-0.1 <sup>a</sup>	Senegal, ricefield	Reynaud and Roger 1978 <sup>c</sup>
Cyanobacteria	0.05-2.8 <sup>a</sup>	0-0.1	Philippines, ricefield	Saito and Watanabe 1978 <sup>c</sup>
Cyanobacteria	0.12-2.6 <sup>a</sup>	0-0.1	India, ricefield	Srinivasan 197g <sup>c</sup>
<i>Aulosira</i> sp.	12.0 <sup>a</sup>	0.48	India, ricefield	Singh 1976 <sup>c</sup>
<i>Gloeotrichia</i> sp.	24.0	0.12	Philippines, ricefield	Watanabe et al 1977 <sup>c</sup>
Green algae	0.06-6.0 <sup>a</sup>	0-0.3	India, ricefield	Mahapatra et al 1971c
Algal biomass	16.0	0.64 <sup>a</sup>	Uzbekistan, ricefield	Muzafarov 1953 <sup>c</sup>
Algal biomass	0-6.0	0-0.24 <sup>a</sup>	Senegal, ricefield	Reynaud and Roger 1978 <sup>c</sup>
<i>Chara</i> sp.	9.0/15.0	0.7-1.2 <sup>b</sup>	India, ricefield	Misra et al 1976 <sup>d</sup>
<i>Chara</i> spp.	2.5-7.5 <sup>b</sup>	0.2-0.6	France, ricefield	Vaquer 1984 <sup>d</sup>
<i>Chara</i> , <i>Nitella</i>	5.0-10.0	0.4-0.8 <sup>b</sup>	India, ricefield	Mukherjy and Laha 196g <sup>d</sup>
<i>Najas</i> , <i>Chara</i>	5.0b	0.4	Philippines, ricefield	Saito and Watanabe 1978 <sup>d</sup>
<i>Marsilea</i>	25.0	2.0 <sup>b</sup>	India, fallow ricefield	Srinivasan 1982d

*continued on next page*

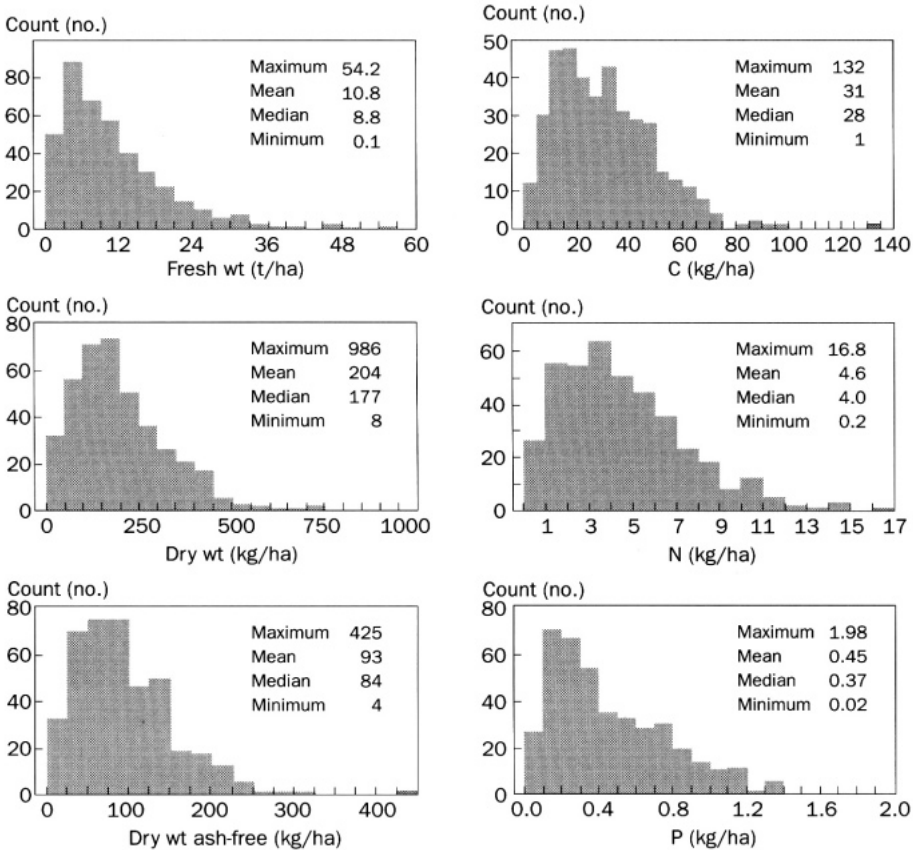
**Table 4.7 continued.**

Nature	Fresh wt (t/ha)	Dry wt (t/ha)	Site	Reference
<b>Total aquatic biomass</b>				
Fallow field	1.0-3.0	0.1-0.24 <sup>b</sup>	Philippines, ricefield	Kulasooriya et al 1981 <sup>d</sup>
Planted field	7.5	0.6 <sup>b</sup>	Philippines, ricefield	Kulasooriya et al 1981 <sup>d</sup>
Fallow field	1.25-2.5 <sup>b</sup>	0.1-0.2	Philippines, ricefield	Inubushi and Watanabe
Planted field	1.25-6.25 <sup>b</sup>	0.1-0.5	Philippines, ricefield	1986
Average	6.0	0.35		
<b>Freshwater bodies and irrigation canals</b>				
<b>Submerged macrophytes</b>				
<i>Ceratophyllum demersum</i>		6.8	USA, temperate lake	Boyd 1974
<i>Hydrilla verticillata</i>		2.5	USA, pond	Stewart 1970
<i>Najas guadalupensis</i>		1.1	USA, pond	Boyd 1974
<i>Nymphoides aquaticum</i>		1.8	USA, pond	Boyd 1974
<b>Floating macrophytes</b>				
<i>Eichhornia crassipes</i>		12.8	USA, pond	Stewart 1970
<i>Myriophyllum verticillatum</i>		2.4	USA, pond	Boyd 1974
<i>Nelumbo lutea</i>		1.0	USA, pond	Boyd 1974
<i>Nuphar advena</i>		0.8	USA, pond	Boyd 1974
<i>Pistia stratiotes</i>		4.6	USA, pond	Boyd 1974
<i>Potamogeton pectinatus</i>		2.2	USA, pond	Boyd 1974
<b>Immersed macrophytes</b>				
<i>Alternaria philoxeroides</i>		7.2	USA, pond	Boyd 1974
<i>Cyperus papyrus</i>		2.7-4.6	Tropical ponds	Gaudet 1974; Stewart 1970
<i>Eleocharis quadrangula</i>		7.2	USA, pond	Boyd 1974
<i>Justicia americana</i>		7.1	USA, pond	Boyd 1974
<i>Sagittaria latifolia</i>		7.3	USA, pond	Boyd 1974
Total aquatic biomass	25 - 350	... <sup>e</sup>	India, irrigation canals	Gupta (undated)
Average		4.5		

<sup>a</sup> Extrapolated on the basis of 4% dry wt. <sup>b</sup> Extrapolated on the basis of 8% dry wt. <sup>c</sup> Cited by Roger and Kulasooriya 1980. <sup>d</sup> Cited by Roger and Watanabe 1984. <sup>e</sup> ... = not available.

dian of about 9 t fw/ha corresponding to 177 kg dw/ha, 28 kg C/ha, and 4 kg N/ha (Roger et al 1989). The highest cyanobacteria biomass recorded (54 t fw/ha) corresponded to 1 t dw/ha, 132 kg C/ha, and 17 kg N/ha.

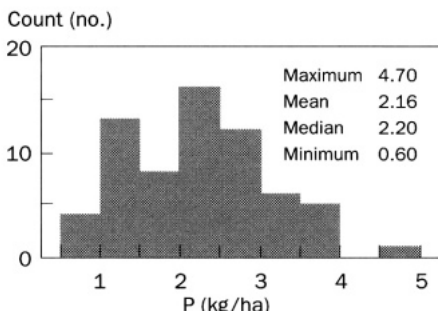
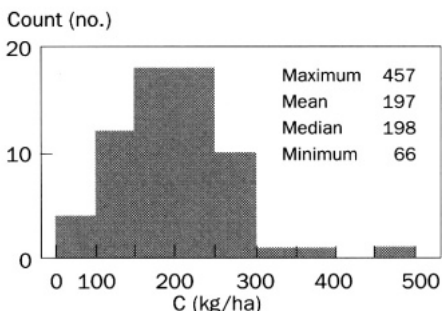
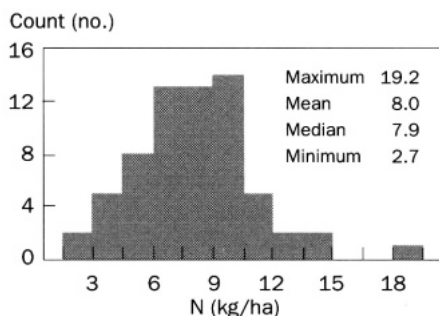
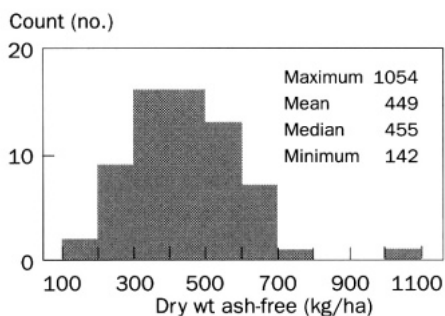
The potential productivity of aquatic macrophytes in ricefields is higher than that of planktonic algae (Table 4.7), but large variations are observed. The biomass of submerged weeds (mainly *Chara* and *Najas*) in 44 plots at the International Rice Research Institute (IRRI) farm averaged 1 t fw/ha (range:



**4.1.** Histograms of 400 estimates of the standing biomass of  $N_2$ -fixing cyanobacteria in 65 plots under various levels of N fertilizer management, when growth was visible to the naked eye (Roger et al 1989).

0.4-3 t) at the end of tillering and 3 t fw/ha (range: 0.2-4.5 t) at rice maturity. It averaged 7.5 t fw/ha (range: 2.7-12 t) in fallow plots. Floating and immersed weed biomass in planted fields averaged 1.7 t fw/ha at tillering (range: 0-4.1 t) (Kulasooriya et al 1981). Weed measurements done by the IRRI Agronomy Department over 9 crops in 3 yr (S. K. De Datta, IRRI, Manila, Philippines, 1989, pers. commun.) ranged from 0.1 to 2.4 t dw/ha and averaged 0.5 t dw/ha. The sum of the weed biomasses harvested at 28 and 56 d after transplanting (DT) in an experimental design of 65 plots subjected to a range of fertilizer treatments (Fig. 4.2) showed a median of 449 kg fw / ha corresponding to 198 kg C/ha and 8 kg N/ha (Roger et al 1989).

In some cases, submerged and floating weeds can develop a very high biomass. Mukherji and Ray (1966, cited by Das 1976) reported that *Chara* and



**4.2.** Histograms of estimates of total biomass of weeds harvested at 28 and 56 d after transplanting in 65 plots under various levels of N fertilizer management (Roger et al 1989).

*Nitella* could develop biomasses of 5-10 t fw/ha on very large areas (about  $5 \times 10^4$  ha) in India when their growth was favored by high temperatures (27-35 °C), a slightly alkaline reaction of water, and clear days with most of the rainfall at night—which allows the muddy water to clear in the day and light to penetrate the water. *Chara* biomasses of 9-15 t fw/ha were reported by Misra et al (1976). In Thaujavur Delta, India, the biomass of *Marsilea quadrifolia* averaged 25 t fw/ha (average of 44 sites) in fallow submerged fields (Srinivasan 1982).

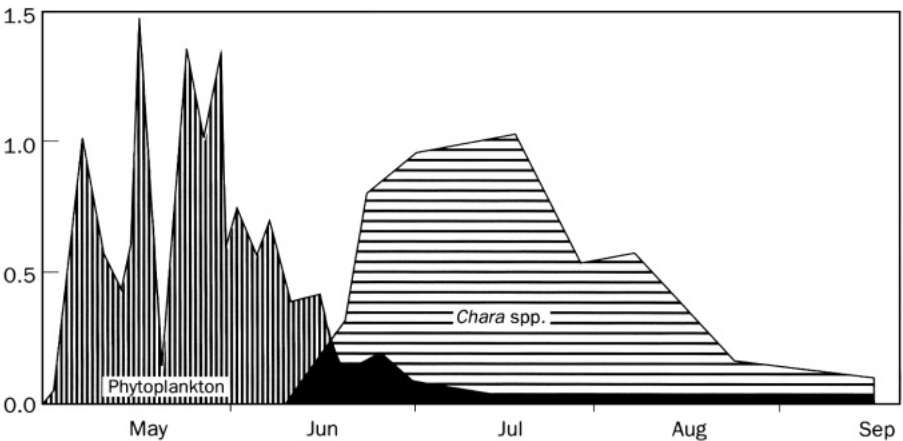
#### **4.3.2. Standing crops in irrigation canals and water sources**

Little is known about standing crops of planktonic algae in water reservoirs. Densities of microcystis blooms observed in Cuttack, India, by Patnaik and Ramchandran (1976) ranged from 7 to 17 ml/liter. Assuming a colonized water layer of 20 cm, this leads to values of 14-30 t fw/ha. It can be assumed that the mass of algal cells per unit area in a culture or a natural ecosystem would not exceed a value that is probably determined by natural shading of the cells. Extrapolation of data from laboratory cultures indicates a theoretical maximum biomass of 2.75 t dw/ha (Roger and Reynaud 1979a).

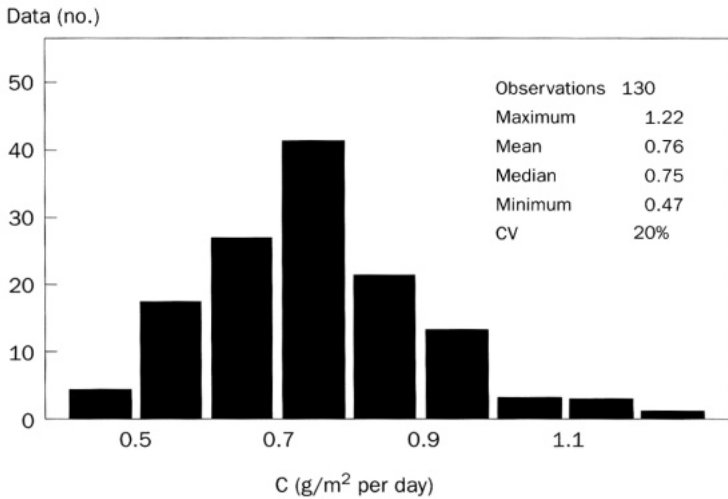
More is known about higher algae and macrophytes. Their very high productivity is frequently mentioned. A classical example is the water hyacinth. In an experiment, two parent plants produced 30 offspring after 23 d and 1,200 after 4 mo (Holm and Yeo 1980). When reproducing vegetatively during the warm season in the USA, water hyacinth has an average doubling time of 12.5 d. *Salvinia*, in open water at the edge of a mat — where it grows faster— can double the area it covers in 8.6 d (Holm and Yeo 1980). Sculthorpe (1967), however, has discussed the mistaken notion that luxuriant submerged or floating macrophytes are unusually productive. The rapid spread of some weeds gives the observer the impression of phenomenal growth, but such vegetation is necessarily buoyant and contains little DM. For example, the amount of DM produced in a *Hydrilla* mat averages only 2.25–4.5 t DM/ha. The plant is 96% water, and mats are usually only about 60 cm thick, because the density of the canopy eliminates all light and reduces growth below that level (Holm and Yeo 1980). In terms of dw, such plants are not very productive compared with other plant communities (Gaudet 1974). Species differ greatly in their inherent ability to produce DM. Large floating plants may have large standing crops. Values of DM above 10 t/ha are commonly encountered in *Eichhornia crassipes*. Other floating and submerged plants normally have standing crops with DM values ranging from 1 to 5 t/ha. More information on productivity and standing crops of aquatic plants can be obtained from Little (1968, 1979).

Biomass measurements and data on the composition of algae and aquatic macrophytes indicate that the N content of spontaneously growing PAB in planted ricefields rarely exceeds 10–20 kg/ha, but might reach 30–40 kg/ha in flooded fallow fields, when large populations of aquatic macrophytes develop.

Productivity (g C/m<sup>2</sup> per d)



4.3. Productivity of the photosynthetic aquatic biomass in a ricefield in France (Vaquer 1984).



**4.4.** Distribution of 130 estimates of average gross primary production during a rice crop cycle in 65 plots on the IRRI farm. Values are the average of 17 or 38 daily estimates during the crop cycle (Roger et al 1989).

#### 4.3.3. Primary production in ricefield floodwater

Reported primary productions of the floodwater community in ricefields are 50-60 g C/m<sup>2</sup> in 90 d (Saito and Watanabe 1978), 70 g C/m<sup>2</sup> in 144 d (Yamagishi et al 1980a,b), and 0.5 to 1 g C/m<sup>2</sup> per day (Vaquer 1984) (Fig. 4.3). Those values correspond to 10-15% of that of the rice crop and are similar to productivity values reported in eutrophic lakes. Measurements at IRRI show that daily primary production may occasionally reach values between 1 and 2 g C/m<sup>2</sup> per day, but in most cases it ranges between 0.2 and 1.0 g C/m<sup>2</sup> per day (Fig. 4.4).

### 4.4. Factors affecting the photosynthetic aquatic biomass

#### 4.4.1. Climatic factors

4.4.1.1. *Light.* Of the habitat factors that affect the composition and dynamics of PAB, the most important is light (Ichimura 1954, Roger and Reynaud 1979a). Its availability influences the activity and the size of the standing crop of primary producers in the floodwater. Its intensity influences the nature of dominant components.

Usually, PAB increases after flooding until the rice canopy decreases light intensity below the compensation point (Ichimura 1954). This may happen any time during the first part of the growth cycle from tillering to panicle initiation (PI) (see sect. 4.5.1). In the tropics, PAB is usually larger during the dry season (DS) when light availability is high than during the wet season

(WS) when solar radiation is lower and there is disturbance by rain. The same applies to photodependent N<sub>2</sub> fixation.

Because light tolerance differs between algal species, light may also have a selective effect on the composition of the flora. Light-adaptation abilities may be roughly correlated with taxonomic groups. Many green algae are adapted to high light conditions, diatoms seem more indifferent to light. Cyanobacteria are generally regarded as low light species (Whitford 1960). Field observations, however, showed that certain cyanobacteria are resistant to and are even favored by high light intensities. In Mali, a dense bloom of *Cylindrospermum* was observed after harvesting, growing under full sunlight (>100 klx at 1300 h) (Traore et al 1978). In India, field growth of inoculated *Aulosira fertilissima* was reported to be better under full sunlight (Singh 1976).

A negative effect of high light intensities on cyanobacteria was reported in Senegal where they developed late in the crop cycle and only under a rice canopy sufficiently dense to protect them from an incident light intensity reaching almost 100 klx at 1300 h daily (Reynaud and Roger 1978). In wet monsoonal and temperate zones, where light intensity is not as high, cyanobacteria may develop from the beginning of the crop cycle during the dry/warm season, but during WS light deficiency may limit their growth (Roger and Kulasooriya 1980). In the Philippines, we consistently observed better growth of cyanobacteria in irrigated fields during DS, when solar radiation is high, than during WS.

In Senegal, Reynaud and Roger (1978) demonstrated the influence of high light intensities on algal successions by incubating submerged unplanted soil under screens to permit the transmission of 100, 60, 22, and 7% of incident sunlight (> 100 klx at 1300 h). After 1 mo of incubation, green algae and diatoms were dominant in the soil placed in full sunlight. Nitrogen-fixing activity was highest in the most shaded soil, where cyanobacteria were dominant.

4.4.1.2. *Temperature.* Both primary production and the composition of PAB are influenced by temperature. Both low and high (> 40°C) temperatures decrease phytoplankton productivity and photosynthetic activity in floodwater (Roger and Kulasooriya 1980). In India, field and pot experiments showed a setback to algal growth during the cold season (Subrahmanyam et al 1965). In Japan, the phytoplankton yield was usually higher on the plain or in warm-temperature districts than in the mountain or cold-temperature districts (Ichimura 1954). When large daily fluctuations of water temperature were observed in the afternoon from June to mid-July, the daytime CO<sub>2</sub> assimilation abruptly declined at temperatures higher than 35 °C (Kurasawa 1956). On the other hand, high temperatures (34-39 °C) in Indian ricefield water were reported to favor the growth of inoculated *Aulosira fertilissima* (Singh 1976). Very high temperatures have a deleterious effect on cyanobacteria and photodependent N<sub>2</sub> fixation (Roger and Reynaud 1979a). Exposure to 42 °C for 50 min reduced the photosynthetic activity of *Nostoc* sp. and *Calothrix* sp. to about 20% of the control (Venkataraman 1964).

Some selective effect of temperature is expected because low temperatures favor eukaryotic algae and higher temperatures favor cyanobacteria. In a ricefield in Japan, *Hydrodictyon reticulatum*, *Spirogyra setiformis*, and *Anabaena oscillarioides* proliferated in summer but were replaced by *Tetraspora gelatinosa*, *Draparnaldia* sp., and *Sphaeroplea annulina* in winter. But water blooms of *Euglena* sp. with different associated species proliferated the year-round without being influenced by water temperature (Mori 1963). In temperate or sahelian zones during DS, a lower temperature at the beginning of the cultivation cycle may favor eukaryotic algae and inhibit cyanobacteria growth (Roger and Reynaud 1977).

As high temperatures are frequently associated with high light intensities, it is important to interpret the effect of these two factors separately in ecological studies.

4.4.1.3. *Desiccation and rewetting.* Soil algae and spore-forming cyanobacteria have a high capacity to withstand desiccation. Resistance to desiccation increases with the dryness of the biotope and can be related to the floristic composition of desiccated soils (Roger and Reynaud 1979a). In a ricefield in Italy, where the dry period was relatively short, N<sub>2</sub>-fixing cyanobacteria made up only about 30% of the algal flora (Materasi and Balloni 1965). In Senegal, where DS lasts about 8 mo, spores of heterocystous cyanobacteria constituted more than 95% of potential flora at the end of the dry period. Eukaryotic algae developing at the beginning of the crop cycle were present primarily because of their introduction by irrigation water (Roger and Reynaud 1976). In Uttar Pradesh, India, a large number of Chlorophyceae occurred in low-lying ricefields where the moist habitat apparently was suitable for their growth. Being more resistant to drier conditions, cyanobacteria occurred in larger numbers in fields at higher elevations (Pandey 1965a,b).

Alternate periods of desiccation and submersion may also influence the quality and quantity of the algal biomass. In ricefields in Japan, algae were more abundant when the soil was waterlogged throughout the year than when it was waterlogged during rice growth only (Okuda and Yamaguchi 1952b). A peak of C and N mineralization occurs during rewetting of desiccated soils; the release of mineral N may decrease the relative competitiveness of N<sub>2</sub>-fixing forms, and the enhanced CO<sub>2</sub> concentration may favor the growth of green algae at the beginning of the cultivation cycle. At the end of the cultivation cycle and as the soil dries, species that form mucilaginous colonies, such as *Nostoc* and *Cylindrospermum* lose their water slowly and can grow for a longer time and even profusely (Pandey 1965a, Traore et al 1978).

4.4.1.4. *Rain.* By increasing water turbidity, rain may limit available light to the extent that photosynthesis and photodependent N<sub>2</sub> fixation are significantly reduced. In the Philippines, we measured in situ acetylene-reducing activity (ARA) before and after a typhoon and observed the suppression of photodependent N<sub>2</sub> fixation, algae being either mixed with soil or washed out of the field (unpubl. data). In nonsubmerged soils, buffeting rains may

mix algae with the top layer of the soil, inhibiting algal growth and photodependent  $N_2$  fixation almost completely (Traore et al 1978). Heavy rains suppressed the development of *Aulosira fertilissima* inoculated in the field (Singh 1976).

4.4.1.5. Wind. Wind may either cause algae to accumulate on one side of the field, decreasing light available for their growth because of mutual shading, or cause dense algal masses to disperse, thus favoring growth.

#### 4.4.2. Soil factors

Among the soil properties, pH is certainly the most important factor determining the composition of the algal flora. In alkaline soils, a heavy algal growth often appears soon after waterlogging, but a poorer growth is observed in acidic soils (De 1939). Soil pH has a selective effect on both the indigenous algal flora and the changes of algal population. The dominant algal species in acidic and alkaline soils often differ because the growth of Chlorophyceae is favored by low pH values and that of cyanobacteria by higher values (Pandey 1965a). In central Sri Lanka, where soils are acidic, eukaryotic algae were dominant in ricefields; in the northern part, where soils are alkaline,  $N_2$ -fixing cyanobacteria were dominant (Kulasooriya and DaSilva 1978). In acidic soils of Kerala (pH 3.6-6.3), application of  $CaCO_3$  increased available N and promoted the growth of  $N_2$ -fixing cyanobacteria; in the untreated plots, predominant algae were Chlorophyceae (Amma et al 1966). In acidic soils where rewetting is generally followed by an increase of pH (Garcia et al 1974), the dynamics of the algal flora composition showed an early growth of Chlorophyceae. Growth of cyanobacteria was delayed until the end of the cultivation cycle, which was attributed *pro parte* to pH increase (Roger and Reynaud 1976, 1977).

Available information on the effects of other soil properties on algal growth deals almost exclusively with cyanobacteria. Next to an alkaline pH, the most decisive factor for their growth was a high available-P content while soil organic matter (OM) content or texture had no clear effect (Okuda and Yamaguchi 1955, 1956; Matsuguchi and Tangcham 1974). In a survey of algal populations in 102 samples of rice soils from the Philippines, India, Malaysia, and Portugal (Roger et al 1987b), a highly significant positive correlation was observed between C content of the soil and 1) total algae and 2) the ratio between total algae and heterocystous cyanobacteria. However, there was no significant correlation between soil C and heterocystous cyanobacteria. A similar trend was observed for N content. These results indicated that soils rich in OM had higher total algal populations and lower relative populations of heterocystous cyanobacteria.

#### 4.4.3. Biotic factors

Biotic factors capable of limiting the growth of phytoplankton in ricefields are pathogens, antagonists, and grazers. Populations of aquatic oligochaetes may limit the growth of macrophytes.

4.4.3.1. *Pathogens*. Certain bacteria, fungi, and viruses (Singh 1973a, Huang 1982) have been shown to be pathogenic to cyanobacteria and algae. In culture, bacteria may lyse vegetative cells of cyanobacteria within 2-10 h, but frequently do not affect heterocysts and spores. Parasitic fungi have a host range limited within a single species. Their seasonal variations are directly correlated with the abundance of the algae on which they occur. However, the presence and the role of parasitic bacteria and fungi in ricefields have not yet been reported. Considering the widespread occurrence of algal viruses and the specific host ranges of the individual strains, it is possible that in natural situations they may be important in determining algal successions and disappearance. Cyanophages were isolated from ricefields in India with a maximum density of 1,000 plaque-forming units/ml. Seasonal variations in their abundance were demonstrated (Singh 1973a).

4.4.3.2. *Antagonists*. Many algae release substances that inhibit either their own growth or the growth of other species, or both. Such substances are probably involved in the succession of species in aquatic ecosystems, but evidence in ricefields is lacking.

Both antagonisticlike effects of phytoplankton on macrophytes and of macrophytes on phytoplankton have been observed. In India, Subrahmanyam et al (1965) reported that cyanobacteria growth suppressed weeds such as *Panicum* sp., *Cyperus* sp., *Hydrolea* sp., and *Ludwigia* sp. In the Philippines, water colonization by submerged weeds suppressed the algal bloom formed after transplanting (Saito and Watanabe 1978). At crop maturity, a negative correlation was also observed between the floating algal biomasses and submerged weed biomasses (Kulasooriya et al 1980), but whether this was due to antagonists or to competition, or both, is still unknown.

Antagonistic effects have also been observed between free-floating and rooted immersed macrophytes. In particular the growth of *Monochoria vaginalis*, *Echinochloa glabrescens*, and *Cyperus difformis* was reduced by 70-100% by *Azolla* (Janiya and Moody 1981b).

4.4.3.3. *Grazers*. Invertebrates such as cladocerans, copepods, ostracods, mosquito larvae, snails, etc. are common grazers of algae in ricefields. They usually appear about 1 wk after the growth of phytoplankton and develop their maximum biomass about 2 wk after the maximum abundance of the phytoplankton (Kurasawa 1956). The development of zooplankton population may cause the disappearance of algal blooms within 1 or 2 wk (Venkataraman 1961) and was reported to prevent the establishment of algal inocula (Hirano et al 1955, Watanabe et al 1955).

Snails form another important group of algal grazers in ricefields, but no work has so far reported their effect on algal successions. In some preliminary experiments, we observed a rapid consumption of laboratory-grown N<sub>2</sub>-fixing cyanobacteria by snails and a heavy biomass of snails (1-1.6 t/ha) in certain ricefields at IIRRI where there was very little algae (unpubl. data).

Detailed information on grazing is presented in sect. 5.3.

4.4.3.4. *Effect of aquatic oligochaetes on weeds.* Kikuchi et al (1975) observed that weed density was lower in plots where tubificids were abundant. In vitro experiments showed that tubificids, by ingesting soil and moving up and down in the plow layer, cause a vertical redistribution of soil particles, concentrating larger particles and weed seeds 3-5 cm under the soil surface in a zone where O<sub>2</sub> concentration is too low for seed germination (Kurihara 1983) (see sect. 5.4).

#### 4.4.4. Agronomic practices

4.4.4.1. *Land preparation and management.* Tillage has a disturbing effect, mainly because of 1) the incorporation of algae and macrophytes (and their spores or seeds) into the soil, and 2) dispersion of clay particles in the submersion water, which decreases available light. After a superficial incorporation of algae into the soil, motile algae are probably more adapted to the recolonization of the submersion water than nonmotile forms. Because tillage increases ammonification, it may favor eukaryotic algae at the beginning of the crop cycle. Midseason tillage, which increases P and Fe availability when available N is low, may favor cyanobacteria growth.

Transplanting, which provides a discontinuous canopy at the beginning of the crop cycle, may favor algal growth compared with broadcasting, which results in an earlier continuous canopy that limits light availability.

Weeding disturbs the ecosystem and can be detrimental to algal growth. The negative correlation between submerged weed biomass and floating N<sub>2</sub>-fixing cyanobacteria observed in a Philippine ricefield (Kulasooriya et al 1980) indicated that the removal of weeds may permit better cyanobacteria growth after a possible growth suppression period. However, no significant correlation between weeding and the standing crop of phytoplankton was observed in Japan (Kurasawa 1956).

Water management directly affects algal growth. In *alkaline lands* of northern India, water impoundment was sufficient to permit the profuse growth of cyanobacteria (Singh 1950, 1961). A similar observation was made in the Philippines (Matalog et al 1978). The rate of irrigation may also affect algal growth. In Australian rice soils, algal development was most profuse where the movement of the irrigation water was reduced and its turbidity was low (Bunt 1961). Alternately drying and rewetting the field throughout the rice germination stage suppressed a detrimental growth of green algae and favored the mass propagation of cyanobacteria (Morar 1968). This practice was also recommended to control grazers that feed on the phytoplankton (see sect. 7.4.4.4.).

4.4.4.2. *Inorganic fertilizers.* The nature and the quantity of fertilizers as well as the application techniques considerably influence the subterranean algal flora.

Combined NPK fertilization has given various results at different sites. The study of 30 acidic ricefields in Senegal indicated that NP fertilization in-

creased total algal biomass and N<sub>2</sub>-fixing algal biomass, the relative N<sub>2</sub>-fixing algal biomass expressed as a percentage of total biomass decreased (Roger and Reynaud 1977). On the contrary, a much larger algal biomass—mostly composed of N<sub>2</sub>-fixing *Gloeotrichia*—was recorded in unfertilized plots (24 t/ha) than in fertilized plots (<3 t/ha) in the Philippines (Watanabe et al 1978). However, surface application of NPK frequently results in profuse algal growth. Usually, planktonic forms (*Chorella* and *Euglena*) develop first and are followed by filamentous forms that persist longer (Singh 1973b). Basal incorporation of fertilizers prevents or reduces such growth, which might cause seedlings to lodge (Roger and Reynaud 1977).

Considered separately, N, P, K, CaCO<sub>3</sub>, and other elements may have differential qualitative and quantitative effects on algal growth. After N, P, K, CaCO<sub>3</sub>, and organic manure treatments, changes in the dominant cyanobacteria species were observed. In particular, *Aphanothece* was predominant when CaCO<sub>3</sub> was applied, *Rivularia* when K was applied, and *Plectonema* when P was applied (Srinivasan 1978).

4.4.4.3. *Nitrogen*. N fertilizer broadcasting generally favors the growth of eukaryotic (non-N<sub>2</sub>-fixing) algae, which results in a total or partial inhibition of N<sub>2</sub>-fixing cyanobacteria. Under N deficiency, N<sub>2</sub>-fixing cyanobacteria are favored by a lack of competitiveness of the other algae and can develop profusely if other environmental factors are not limiting. When N fertilizer is applied, N<sub>2</sub>-fixing cyanobacteria can use it for their growth, but have to compete with non-N<sub>2</sub>-fixing cyanobacteria and eukaryotic algae. Little is known about how the nature and the concentration of inorganic N affect this competition. But the selective action and inhibitory effect of N fertilizers on N<sub>2</sub>-fixing cyanobacteria were demonstrated in many experiments under a wide range of agroecological conditions. In the Philippines, Yoshida et al (1973) observed that N fertilizer increased algal growth, and that there were more cyanobacteria when no N fertilizer was applied. Okuda and Yamaguchi (1952), in a 20-mo study of the algal flora in soils treated with different N fertilizers, found that N<sub>2</sub>-fixing forms became abundant only in the unfertilized control. In Myanmar, the total algal biomass increased and green algae were dominant in soils treated with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> or calcium cyanamide, whereas N<sub>2</sub>-fixing *Anabaena* was dominant in the controls without N fertilizer (Than Tun 1969). In India, Subrahmanyam et al (1965) also observed that, after (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> application, *Spirogyra* sp. and *Euglena* sp. were so abundant that rice farmers had to interplant their crop to prevent the algae from smothering the rice plants. In Australia, a survey of rice soils showed that although N<sub>2</sub>-fixing cyanobacteria were isolated from almost all the soil samples, their presence in the fields was not apparent. That was attributed to the application of heavy dressings of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and the addition of CuSO<sub>4</sub> to irrigation water (Bunt 1961).

In situ measurements of the photodependent N<sub>2</sub>-fixing activity confirm the inhibitory effect of N fertilizers on N<sub>2</sub>-fixing cyanobacteria; however, inhibition is often partial (see sect. 4.6.1), and decreases during the growth cycle because of the uptake of N by plants, especially at later growth stages.

Compared with N fertilizer broadcasting, deep placement neither prevents the dense growth of green algae observed with surface broadcasting, nor does it inhibit cyanobacteria growth and photodependent N<sub>2</sub> fixation (Roger et al 1980).

4.4.4.4. *Phosphorus*. Requirements in P for optimal algal growth differ considerably between species when no other external factor is limiting. However, no conclusions could be made about the relationship between I requirements and taxonomic groups (Roger and Kulasooriva 1980). The P concentration in algae varies widely depending on whether or not they are growing under P-deficient conditions. Cyanobacteria assimilate more P than they require and store it as polyphosphate, which can be used under P-deficient conditions. In rice soils, which are often P-deficient, P fertilizer and P-supplying manure enhanced algal growth, N<sub>2</sub> fixation (Arora 1972, De and Sulaiman 1950, De and Mandal 1958, Srinivasan 1978, Than Tun 1969), and species diversity (Marathe 1963). Application of P together with CaCO<sub>3</sub> frequently increased growth of N<sub>2</sub>-fixing cyanobacteria (Araragi et al 1978, Ishizawa et al 1975, Yamaguchi 1975), particularly in poor soils (Shiori et al 1950). However, it was also reported to cause N loss in fertile soils that had frequently received organic fertilizers (Shioiri et al 1944), probably because an enhanced algal growth in N-rich soils favors losses by NH<sub>3</sub> volatilization (see sect. 4.6.4)

4.4.4.5. *Calcium*. Calcium is not a limiting factor for algal growth in ricefields. The effect of liming on algal flora is more related to pH increase than to Ca availability. By increasing soil pH, liming favors cyanobacteria. Laboratory incubations of acidic soils in light showed that cyanobacteria growth and N<sub>2</sub> fixation did not occur unless pH was increased by liming (De 1936). Roger and Kulasooriya (1980) cite seven references reporting that the addition of CaCO<sub>3</sub> in rice soils enhances cyanobacteria growth and N<sub>2</sub> fixation. But in experimental ricefields of Japan, superphosphate favored cyanobacteria growth more than Ca because the increase of the limiting pH in the floodwater, obtainable by liming, was also achieved by the growth of algae once P deficiency was overcome (Okuda and Yamaguchi 1956).

4.4.4.6. *Other elements*. Applied singly or together with N and P, K either had no effect or reduced algal growth (De and Sulaiman 1950, Mahapatra et al 1971). Magnesium sulfate also reduced algal growth when used in the absence of phosphate (De and Sulaiman 1950). Many other elements (Na, S, Cl, Co, Zn, Cu, etc.) are required for optimum growth of algae, but their ecological implications as limiting factors or as factors affecting the composition of the algal community in ricefields have not been demonstrated.

4.4.4.7. *Organic manure*. The effects of organic fertilizers on algae and aquatic weeds seem to vary. Both favorable and inhibitory effects have been reported.

The incorporation of crop residues or green manures may stimulate planktonic algae growth during the early stages of rice development. It was observed in California (Chapman et al 1972) and in Australia (Noble and Happey-

Wood 1987). In both cases, algae growing as a scum were detrimental to rice directly seeded in fields where straw had been incorporated or legumes were previously grown. In Japan, an earlier and more abundant growth of *Pithophora* was observed in plots where green manure was applied compared with control and plots receiving inorganic fertilizer (Yamasaki and Saeki 1985).

By immobilizing N fertilizer, OM incorporation or application may favor the growth of N<sub>2</sub>-fixing cyanobacteria. That was observed in India after the incorporation of green manure (Marathe 1964). Venkataraman (1968) reported that farmyard manure enhanced cyanobacteria growth when it was applied together with (NH<sub>4</sub>)<sub>2</sub>SO<sub>5</sub> and P. In Japan, the dominance of green algae was observed in chemically fertilized plots with and without organic manures, but N<sub>2</sub>-fixing cyanobacteria were recorded only in the plots with organic manure (Kikuchi et al 1975). Surface application of straw has been reported to stimulate N<sub>2</sub>-fixing cyanobacteria growth and phototrophic ARA (Matsuguchi and Yoo 1981).

On the other hand, OM incorporation has also been reported to temporarily reduce algal growth, especially during the active decomposition of the OM. Compost applied with superphosphate suppressed N<sub>2</sub>-fixing cyanobacteria, which was interpreted as a toxic effect of sulfate reduction byproducts (El-Fadl et al 1964). Roger and Kulasooriya (1980) cite five reports of negative effects of organic manures on cyanobacteria in soil that had been inoculated with these organisms.

The effects of organic manure on algal flora vary with its nature. A change in the N<sub>2</sub>-fixing dominant species was observed after application of compost and farmyard manure, but not with green leaf manure (Srinivasan 1978). Sugar factory waste water that pollutes irrigation water was reported to preferentially enhance homocystous cyanobacteria growth (*Oscillatoria*, *Spirulina*, *Lyngbia*) (Jutono 1973).

4.4.4.8. *Pesticides*. Herbicides and algicides affect PAB directly. Insecticides affect it mostly indirectly through their effects on primary consumers. Those aspects are developed in sect. 6.3.

## 4.5. Dynamics of photosynthetic aquatic biomass during the crop cycle

From reports concerning the variations of the algal flora along the cultivation cycle, it appears that maximum biomass could develop any time and is mainly related to climatic conditions. Developments of dense algal blooms just after transplanting, due to fertilizers or plowing, or both, and a high light availability, have been reported (Saito and Watanabe 1978). In ricefields in Japan, the maximum algal biomass was observed about 2 wk (Kurasawa 1956) or 1 mo (Ichimura 1954) after transplanting. Subsequent decrease of the biomass was related to the consumption by grazers (Kurasawa 1956) and to light deficiency under the rice canopy (Ichimura 1954, Kurasawa 1956). In the Ukraine,

maximum algal growth was observed just before tillering (Prikkhod'Kova 1968). In ricefields in Senegal, maximum biomass developed between tillering and PI (Roger and Reynaud 1976). In dryland ricefields in India, a similar evolution was observed. In wetland fields, however, the density of the biomass reached maximum a little later than in Senegal (Gupta 1966). In the Philippines, during DS, algal density was highest just after heading of the rice crop. During WS, development was maximum after harvesting (Watanabe et al 1977b, 1978), probably because of an increase in light availability.

Algal populations appear to be highly susceptible to environmental changes and exhibit rapid qualitative and quantitative variations along the crop cycle. Studies describe algal successions in ricefields of India (Gupta 1966), Senegal (Reynaud and Roger 1978, Roger and Reynaud 1976, 1977), and the Philippines (Grant et al 1986).

A study in Allahabad district, India, by Gupta (1966) compared dryland fields planted to an early-maturing rice variety and wetland fields planted to a late-maturing variety.

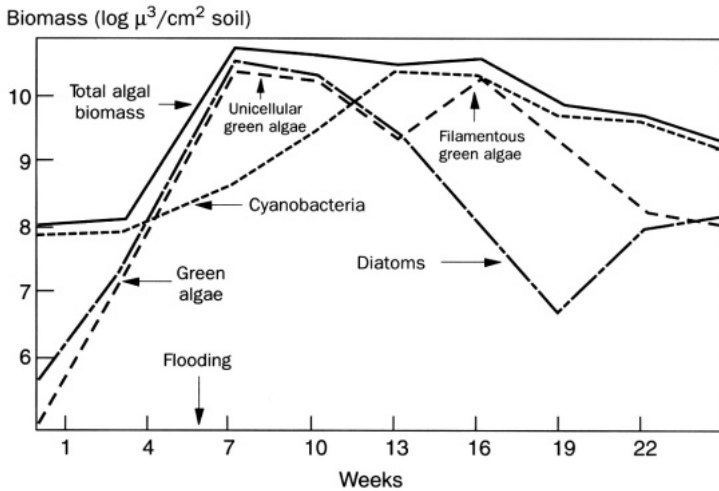
- In the dryland area, algae began to grow 1 mo after the first monsoon rain; the quality was poor and the quantity was low. The algal community was dominated by *Spirogyra* associated with *Anabaena*. During the next third of the cultivation cycle, algal biomass became abundant with a dominant growth of *Anabaena*. During the last third of the cycle, more species were observed and the N<sub>2</sub>-fixing genera were well represented by *Anabaena*, *Scytonema*, and *Nostoc*.
- In the wetland area, fresh water remained muddy 2 mo after the beginning of the monsoon and no algae grew. *Anabaena* was the first cyanobacteria genus to develop, 2 wk after transplanting. During the next 2 mo, abundant, mixed algal flora were observed. N<sub>2</sub>-fixing forms (*Scytonema*, *Aulosira*, *Nostoc*) were associated with *Chara* sp. and filamentous green algae. At the end of the cycle, eukaryotic algae were disintegrating and *Scytonema* was dominant.
- In both soils, N<sub>2</sub>-fixing cyanobacteria were present from the beginning of the algal community and became abundant in the second half of the cycle.

The main differences between these two types of ricefields were

- a later growth of algae in wetland soils,
- a well-marked dominance of cyanobacteria in dryland soils, and
- a mixed community of eukaryotic algae and cyanobacteria in wetland soils.

The author concluded that the difference in growth between wetland and dryland fields was associated with ecological conditions, and the overall succession was controlled chiefly by seasonal variations.

The dynamics of the algal flora were studied in 40 ricefields in Senegal by Reynaud and Roger (1978) and Roger and Reynaud (1976, 1977). In this area, soils are acidic: average pH is 5.0 at the beginning of rice cultivation and 6.2



4.5. Dynamics of algal biomass in a ricefield in Senegal (Roger and Reynaud 1976).

after 2 mo of submersion. The rainy season is short (15 Jul-15 Nov) and ricefields are dry the rest of the year. High light intensities reaching 100 klx occur throughout the year. The following scheme for algal successions in this area was proposed.

During the early part of the cultivation cycle (planting and tillering), the algal biomass increased and consisted mainly of diatoms and unicellular green algae (Fig. 4.5). From tillering to PI, the algal biomass reached its highest values, and filamentous green algae and non-N<sub>2</sub>-fixing cyanobacteria were dominant. After PI, the total biomass decreased. If the plant cover was sufficiently dense, heterocystous cyanobacteria developed; if it was thin, filamentous green algae and homocystous cyanobacteria remained dominant.

The following interpretation of algal flora successions was proposed. At the beginning of the cultivation cycle, environmental conditions were

- a low soil pH, which favored the growth of Chlorophyceae but not of cyanobacteria,
- an absence of plant cover and a resulting high light intensity reaching the water that was also favorable for Chlorophyceae and diatoms but unfavorable to cyanobacteria, and
- high levels of CO<sub>2</sub> and available N, caused by soil remoistening, favorable to Chlorophyceae.

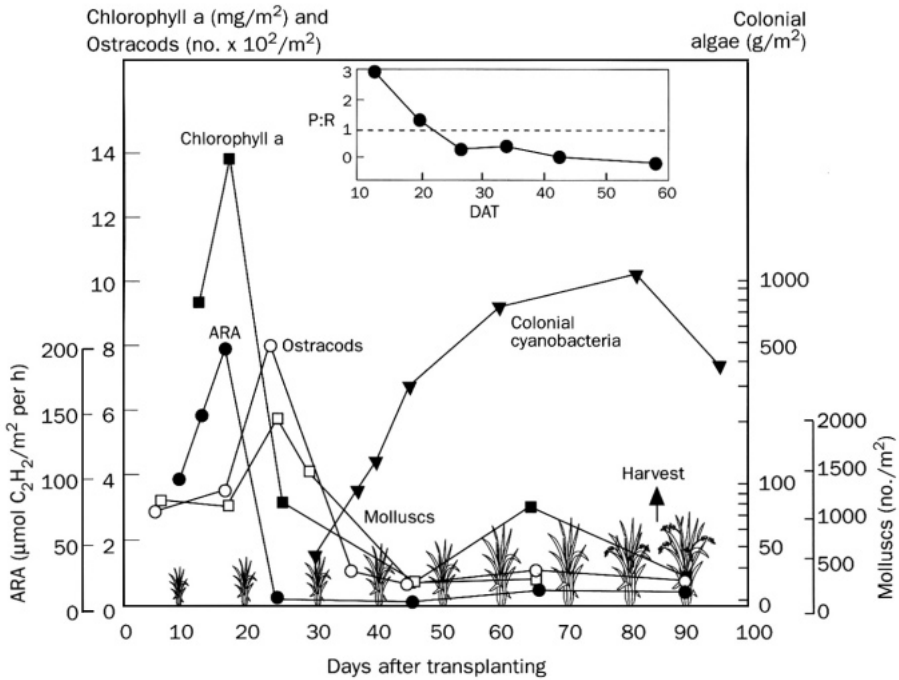
During the cultivation cycle, a decrease in light intensity and N level related to rice growth and an increase in pH favored cyanobacteria growth. The nonevolution of algal flora composition under a weak plant cover indicated the important role of light in regulating algal composition.

It is clear that the proposed interpretation of algal succession was incomplete (i.e., nutrition and competition may affect the sequence of the algae groups) and should be considered characteristic of a definite geographic zone having acidic soils, high light intensities, and a semiarid climate (Reynaud and Roger 1978, Roger and Reynaud 1976, 1977). However, the same kind of algal succession was described in the Kuban area (Morar 1968) and the Philippines (Pantastico and Suayan 1973), where diatoms predominated during land preparation for transplanting, followed by green algae as the plants grew, and cyanobacteria just before and during harvest. Also in Japan, diatoms and unicellular green algae developed at the beginning of the crop cycle, followed by filamentous green algae. But the dominant alga at the end of the cycle was *Trachelomonas* and cyanobacteria were never dominant (Kikuchi et al 1975), probably because of the high level of N fertilizer used and the low temperature, both of which are unfavorable to cyanobacteria.

A frequent observation is that N<sub>2</sub>-fixing cyanobacteria rarely become dominant at the beginning of the cultivation cycle (Roger and Kulasooriya 1980). In a lysimeter experiment in Japan, green algae developed their maximum biomass at the beginning of the cultivation cycle, and then decreased; cyanobacteria reached their maximum abundance in the middle of the cycle and became dominant at the end (Suzuki 1967). A similar observation was made in Varanasi, India, where eukaryotic algae developed first. The appearance of cyanobacteria was earlier in nonfertilized plots than in fertilized ones (Singh 1978). However, the growth of a dense N<sub>2</sub>-fixing bloom was reported in Mali at the earliest stage of rice growth (Traore et al 1978). Nitrogen-fixing forms were observed throughout the growth cycle in Sri Lanka; *Nostoc* and *Anabaena* appeared during the early stages and persisted throughout the cycle, *Gloeotrichia* and *Rivularia* appeared during the later part (Thirukkanasan et al 1977).

By combining the data of Pantastico and Suayan (1973), Watanabe et al (1978), Saito and Watanabe (1978), and their own observations, Grant et al (1986) presented the following generalized succession of the predominant dry season flora and fauna from nonfertilized ricefields of the Philippines (Fig. 4.6).

- Eukaryotic algae develop first and are soon succeeded, albeit briefly, by fast-growing noncolonial heterocystous cyanobacteria about 2-3 wk after transplanting. The chlorophyll a concentration of the floodwater and floodwater/soil interface peaks as a result of the cyanobacteria bloom, but rapidly subsides to a basal level (0-3 mg chlorophyll a/m<sup>2</sup>) thereafter. The decline in the initial cyanobacteria bloom is related to grazing by ostracods and molluscs. The collapse of the ostracod population quickly follows that of the cyanobacteria.
- About 4 wk after transplanting, a population of floating and slow-growing mucilaginous, colonial cyanobacteria that are resistant to grazing develops and reaches a maximum biomass before harvest.



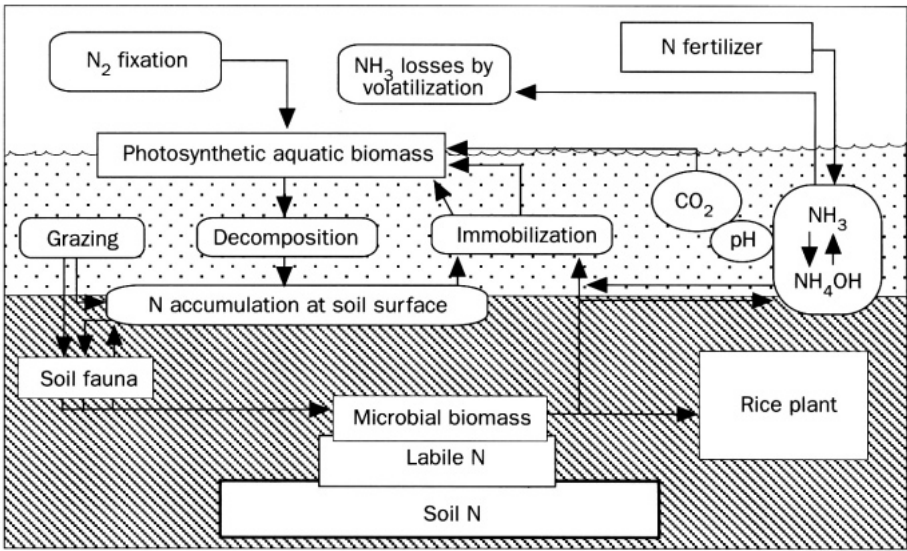
4.6. Generalized fluctuations of algae, acetylene-reducing activity (ARA), and grazers in floodwater of unfertilized lowland ricefields (Grant et al 1986). Production: respiration (P:R) calculated from Ichimura (1954).

- The recruitment rate of molluscs depends upon the previous field condition. After a wet fallow, they may rapidly attain densities detrimental to cyanobacteria growth. They subside with the disappearance of algae, which also signals the change from an autotrophic to a heterotrophic stage indicated by O<sub>2</sub> productivity.
- Primary production typically exceeds community respiration (P:R >1) over the first month of rice cultivation (inset Fig. 4.6), which leads to the buildup of OM and a decrease of the ratio to below 0.1.
- Coincident with the initial cyanobacteria bloom, which is not usually resistant to grazing, is a peak of N<sub>2</sub> fixation activity. Nitrogen fixation associated with mucilaginous, colonial cyanobacteria develops later on, at a lower value, but lasts longer.

#### 4.6. Photosynthetic aquatic biomass and nitrogen cycle

Figure 4.7 summarizes the major aspects of the N cycle involving PAB and its activities. It includes

- photodependent biological N<sub>2</sub> fixation,
- immobilization of N from the floodwater and the surface soil,



4.7. Role of the photosynthetic aquatic biomass in N cycling in wetland ricefields (Roger 1987).

- N recycling in relation to spontaneous death or grazing, decomposition, N accumulation at soil surface, and translocation to deeper soil by soil fauna,
- contribution to the replenishment of the soil microbial biomass,
- provision of N to the rice plant, and
- induction of N losses by NH<sub>3</sub> volatilization through increasing floodwater pH.

#### 4.6.1. Photodependent biological N<sub>2</sub> fixation

Photodependent N<sub>2</sub>-fixing microorganisms in wetland ricefields consist of photosynthetic bacteria, free-living cyanobacteria, and symbiotic cyanobacteria in *Azolla*. *Azolla* is an aquatic fern, which harbors the symbiotic N<sub>2</sub>-fixing cyanobacteria *Anabaena azollae*. Spontaneous development of *Azolla* in ricefields is much less frequent than that of free-living cyanobacteria, which are ubiquitous. Fields usually need to be inoculated with *Azolla* when it is to be used as green manure (see sect. 7.3).

##### 4.6.1.1. Methods for estimating photodependent N<sub>2</sub> fixation in ricefields.

All available methods (biomass measurement together with estimation of N derived from N<sub>2</sub> fixation, ARA measurement, <sup>15</sup>N incorporation, <sup>15</sup>N dilution, and the difference in natural <sup>15</sup>N abundance) have been used to estimate photodependent biological N<sub>2</sub> fixation (BNF) in ricefields. However, ARA measurement, despite recognized limitations, is still the most popular method and was used in about two-thirds of the 38 quantitative BNF studies related

to rice published since 1985 (Roger and Ladha 1992). Methods where composite or standardized samples collected in situ are incubated under controlled laboratory conditions have been developed to overcome limitations due to the heterogeneous distribution of photodependent  $N_2$  fixers and the greenhouse effect that develops in enclosures used for incubation in situ. Using 400 groups of four- and five-replicated ARA measurements in plots with various agronomic practices, Roger et al (1991a) drew general conclusions on sampling densities and replications needed for a given level of accuracy. The distribution of photodependent ARA approximates a log-normal pattern. On the average, three replicated plots are needed to show significant differences between means whose ratio is  $> 5$ . Ten plots are needed for a ratio of 2. Interplot variability cannot be reduced, but ARA values integrated for the crop cycle are less variable than daily values. Using composite samples markedly reduces intraplot variability. In a  $16\text{-m}^2$  plot, composite samples of 10 cores (2 cm diam) each have a  $CV = 20\%$ .

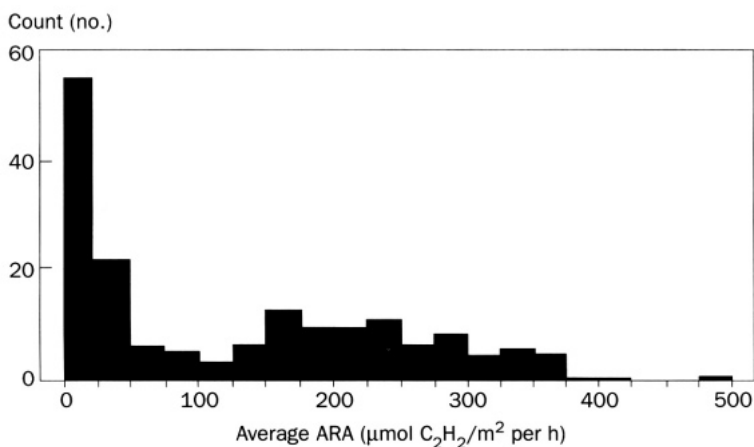
$^{15}\text{N}$  incorporation was used for short-term studies to assess  $N_2$  fixation by various agents, to identify active sites in soil or rice plant, and to establish the  $C_2H_2/N$ , conversion factor in cyanobacteria and *Azolla* (Watanabe and Roger 1985b, Eskew 1987).

The  $^{15}\text{N}$  dilution method is attractive because one sampling can provide an estimate of BNF in plants integrated over time. With aquatic  $N_2$  fixers, problems caused by fast changes in  $^{15}\text{N}$  enrichment over time, which result in large errors in percent N derived from the atmosphere (Ndfa) estimation (Witty 1983), can be solved by the sequential addition of  $^{15}\text{N}$  in water (Kulasooriya et al 1988). But with cyanobacteria, the N level in water sufficient for growth of nonfixing control algae may inhibit cyanobacteria growth directly or through competition (Roger and Ladha 1992).

The difference in natural  $^{15}\text{N}$  abundance ( $\delta^{15}\text{N}$ ) was used to estimate Ndfa in *Azolla* (Yoneyama et al 1987).

4.6.1.2. *Estimates*. Photosynthetic bacteria have been recorded in rice soils, but their contribution to the N input is usually low (Roger and Watanabe 1986). Greenhouse and field experiments show that straw application may significantly increase populations and photodependent  $N_2$ -fixing activity of photosynthetic bacteria and cyanobacteria (Ladha and Bonkerd 1988). Quantitative estimates of BNF in field experiments with straw are not available, but a few semiquantitative data and laboratory data suggest that straw might increase RNF by 2-4 kg N/t straw applied. Part of the increase is due to photosynthetic bacteria.

Nitrogen fixation by cyanobacteria has been almost exclusively estimated from ARA. Data published before 1980 vary from a few to 80 kg N/ha per crop (mean 27 kg) (Roger and Kulasooriya 1980). Figure 4.8 presents the distribution of 180 estimates of average photodependent ARA during a crop cycle in 180 experimental plots under various management practices (Roger et al 1988). The histogram is bimodal, showing the combination of a log-normal



**4.8.** Distribution of 180 estimates of average ARA during a crop cycle in experimental plots under various management practices (Roger et al 1986).

distribution on the left of the graph, corresponding to plots where ARA was inhibited (mostly by N fertilizer application) and a bell-shaped distribution on the right side of the graph, corresponding to plots where a significant ARA developed. Extrapolated values (assuming  $C_2H_2/N_2 = 4$ ) ranged from 0.2 to 50 kg N/ha per crop and averaged 20 kg in no-N control plots, 8 kg in plots with broadcast urea, and 12 kg in plots where urea was deep-placed. ARA was negligible in 75% of the plots where urea was broadcast (Roger et al 1988).

Biomass measurements provide a rough estimate of the  $N_2$ -fixing potential of cyanobacteria because they bloom only when the photic zone is depleted of N, therefore most of their N can be assumed to originate from BNF. Inubushi and Watanabe (1986) estimated that cyanobacteria in  $^{15}N$ -labeled plots had about 90% Ndfa. However, biomass measurement may underestimate  $N_2$  fixed, because the turnover of the algal biomass is not taken into account. Studies of cyanobacteria blooms and crusts (IRRI 1986, Roger et al 1985a,b; 1987a,b) indicate that 1) a visible growth of cyanobacteria usually corresponds to less than 10 kg N/ha, 2) a dense bloom may correspond to 10-20 kg N/ha, and 3) higher values (20-45 kg N/ha) are recorded only under artificial conditions as in experimental microplots or in cyanobacteria soil-based inoculum production plots. More than two blooms of  $N_2$ -fixing cyanobacteria rarely occur during a crop cycle. Therefore 20-30 kg N/ha per crop seems a reasonable estimate of photodependent BNF when a dense cyanobacteria growth is visible.

BNF by *Azolla* has usually been estimated from biomass measurement and the assumption that most of *Azolla* N originates from BNF. The N potential of *Azolla* was summarized by Roger and Watanabe (1986) from data obtained mostly in experimental plots. The N content in maximum standing crops ranged from 20 to 146 kg/ha and averaged 70 kg/ha (n = 17; CV = 58%). Nitrogen-fixing rate ranged from 0.4 to 3.6 kg N/ha per day and averaged 2 kg N/ha per day (n = 15, CV = 47%). In a 4-yr field trial at 37 sites in 10 countries, productivity was lower than in experimental plots (Watanabe 1987). Biomass was 5-25 t fw/ha (10-50 kg N/ha) for *Azolla* grown before or after transplanting (average 15 t/ha or 30 kg N/ha). Recent experiments focus on Ndfa determination. Using the <sup>15</sup>N dilution method with *Lemna* and *Salvinia* as nonfixing controls, Watanabe and Talukdar, and Kumarsinghe (unpubl. data cited by Eskew 1987) estimated that 80-85% *Azolla* N was Ndfa. Using similar controls and applying <sup>15</sup>N-labeled urea at 3-d intervals for 14 d, Kulasooriya et al (1988) found 51-61% Ndfa and BNF of 10-14 kg N/ha in 14 d. Using the <sup>15</sup>N method, Yoneyama et al (1987) estimated that 59-99% of N of the strains tested was Ndfa. *Azolla filiculoides*, freed from its symbiont and the *Lemna* control, had a similar δ <sup>15</sup>N, which was not influenced by the water level (flooded or saturated soil).

4.6.1.3. *Relative importance of photodependent BNF.* Pot studies comparing N balance in the presence and the absence of light in the water and surface soil provide an estimate of the contribution of photodependent BNF to total BNF in wetland soils (App et al 1986, Santiago-Ventura et al 1986, Singh and Singh 1987, Trolldenier 1987). In a 4-crop experiment comparing the effects of soil exposure to light, presence of rice, and flooding in nonfertilized plots, N gains ranged from 78 to 103 mg/crop per pot in fallow pots not exposed to light and from 243 to 277 mg/crop per pot in planted pots exposed to light (Singh and Singh 1987). Table 4.8 presents a bibliographic analysis of N bal-

**Table 4.8. Effect of various factors on N balance (adapted from Roger and Ladha 1992).**

Factor	Level	Observations (no.)	Mean (kg N/ha per crop)	Standard deviation	Significance of the difference <sup>a</sup>
Inorganic N	None	166	29.7	25.4	**
	Added	45	4.0	47.6	
Rice plant	Planted	193	26.5	30.7	**
	Unplanted	18	-0.5	46.2	
Light (all data)	Light	197	25.0	33.9	ns
	Dark	14	13.2	13.8	
Light (no N applied)	Light	152	31.2	25.7	*
	Dark	14	13.2	13.8	

<sup>a</sup> Level of significance of the difference between the two levels tested for each factor: ns = not significant, \* = p<0.05, \*\* = p< 0.01.

ance in rice soils showing that, in the experimental designs used, photo-dependent BNF contributed 50-70% to total BNF (Roger and Ladha 1992).

#### **4.6.2. Nutrient immobilization and recycling**

The PAB prevents N losses by immobilizing floodwater N and returning it as organic N into the soil. Immobilization is obvious but poorly documented. Shiori and Mitsui (1935) measured an immobilization rate of 10-26% of N applied in pots. Using a gas lysimeter, Vlek and Crasswell (1979) estimated that, 3 wk after N fertilizer application, immobilization in the algal biomass of N from fertilizers was 18-30% for urea and 0.4-6% for  $(\text{NH}_4)_2\text{SO}_4$ . These results were confirmed by  $^{15}\text{N}$  experiments showing immobilization of 18-41% of N from urea applied in the floodwater 3 wk before (Vlek et al 1980). When  $^{15}\text{N}$ -labeled  $(\text{NH}_4)_2\text{SO}_4$  was incorporated into the puddled layer, < 5% was immobilized (Inubushi and Watanabe 1986).

#### **4.6.3. Contribution to the microbial biomass and available N**

Rice is heavily dependent on N mineralized from soil OM, but only a small fraction of total soil N is easily available to the crop. The chloroform fumigation method (Jenkinson and Ladd 1981) has shown that microbial biomass is a major channel through which nutrients are transferred to crop plants. Marumoto (1984) found that in oven-dried and rewetted rice soils, 66% of the N mineralized during 28 d of incubation came from the newly killed (chloroform-fumigated) microbial biomass.

Estimates of microbial biomass in wetland rice soils (Marumoto 1984, Hasebe et al 1985) show higher ratios of microbial biomass C to total soil C (4-8%) than reported for upland arable lands (Jenkinson and Ladd 1981). Total microbial biomass may be larger in flooded soils because of the development of the aquatic microbial community, especially microalgae.

Early experiments in drums at IRRI showed that flood fallow promotes N accumulation. After 24 consecutive crops, N in soils subjected to flood fallow was 0.183-0.185%; that in dry fallow was 0.118-0.126%. After 10 successive crops without N fertilizer application, rice grown on flood fallow yielded more (112-140 g/drum) than rice grown on dry fallow soils (77-93 g/drum) (IRRI 1977).

As a result of the activities of PAB, the consumer populations, and the microbial communities, N accumulates at the soil surface. Reported values range from a few (App et al 1984) to 35 kg N/ha per crop (Ono and Koga 1984). Nitrogen accumulating at the surface of wetland rice soils may come from 1) the atmosphere, through  $\text{N}_2$  fixation; 2) the floodwater, through immobilization by the aquatic biomass; and 3) the soil, after absorption by rooted plants or ingestion by invertebrates. However, the process is mostly photodependent, as demonstrated by field experiments with a control placed in the dark (App et al 1984) or a control that prevents exchanges between surface soil and deeper soil. Ono and Koga (1984) measured the accumula-

tion of 35 kg N/ha per crop under normal field conditions and of 26 kg N/ha per crop when surface soil was isolated from deeper soil by placing it in petri dishes.

Chlorophyll-like substances accumulate at the soil surface in parallel with microbial biomass N (Watanabe and Inubushi 1986). A positive correlation between chlorophyll-type compounds and mineralizable N or soil N-supplying ability (Inubushi et al 1982, Wada et al 1982, Watanabe and Inubushi 1986) indicates that photosynthetic biomass contributes significant quantities of available N and plays an important role in maintaining the fertility of wetland soils. Wada et al (1982) concluded that fields of higher natural fertility will possess greater concentrations of chlorophyll a or chlorophyll-like substances. Watanabe and Inubushi (1986) observed that microbial biomass measured by chloroform fumigation increased at the soil surface and decreased in the puddled layer during flooding. The microbial biomass N in the upper 1-cm soil layer accounted for 10-20% of that in the 0-15-cm layer. This also indicates a significant direct or indirect contribution of the aquatic communities to the total microbial biomass and the nutrition of rice.

It thus appears that a significant part of the fertility of wetland soils results from the activity of a PAB of a few hundred kilograms per hectare, which allows the recycling of nutrients in the ecosystem through decomposition and grazing by the microfauna.

#### **4.6.4. Nitrogen losses by volatilization**

The poor efficiency of N fertilizer use by rice is partly due to N losses by  $\text{NH}_3$  volatilization, which range from 2 to 60% of applied N (Fillery et al 1984). Water pH is a major factor in determining the rate and extent of losses (up to pH 9,  $\text{NH}_3$  concentration increases by a factor of 10 per unit increase in pH). Therefore, aquatic photosynthetic organisms play a key role in  $\text{NH}_3$  volatilization. They deplete  $\text{CO}_2$  in floodwater during the day and partly replenish it at night through respiration, thus causing diurnal changes in floodwater pH, which may reach values as high as 10 by midday and decrease by 2-3 units at night (Mikkelsen et al 1978). Practices decreasing algal growth, such as Cu application (Mikkelsen et al 1978) and deep-placement of N fertilizer (Zhi et al 1984), decrease diurnal increase of pH and N losses.

Fillery et al (1986) estimated the photosynthetic biomass in fields where N losses from fertilizer applied 18 d after transplanting were evaluated. One week after fertilizer application, a limited and uneven growth of algae (100-300 kg fw/ha) was observed in N-treated plots where pH at noontime ranged from 7.8, where no algal growth was visible, to 10.5 in the vicinity of algal colonies. Despite the low algal biomass, significant N losses (30-40%) occurred, suggesting that large algal populations are not required to increase floodwater pH to levels that support rapid N losses. Apparently, the most unfavorable situation seems to be at the beginning of the crop cycle, when there is almost no canopy. The resulting high light availability permits a high photosynthetic

activity of a low algal biomass sufficient to induce a significant pH increase in the floodwater but not to limit N loss through immobilization. Losses from application around PI are generally lower (10-15% of applied N) because 1) the larger canopy reduces wind speed at the water surface, 2) the canopy reduces photosynthetic activity in the floodwater and therefore the maximum value of the floodwater pH, and 3) N uptake by the crop is more rapid.

Measurements at IRRI showed that submerged macrophytes (*Chara*, *Najas*, etc.) significantly increased floodwater pH, whereas it was fairly stable under floating macrophytes *Azolla* and *Lemna*. Such result indicates that there is a potential for combined use of *Azolla* and chemical N.

## 4.7. Decomposition and availability of photosynthetic aquatic biomass nutrients to rice

### 4.7.1. Mechanisms of nutrient release

Living aquatic plants continuously excrete appreciable amounts of dissolved OM, including soluble nutrients (Kristritz 1978). Laboratory experiments have frequently shown that cyanobacteria liberate large portions of their assimilated nitrogenous substances; however, the large amounts recorded may be a methodological artifact due to osmotic shock in resuspending the cells or to physical damage of the algal material. No information on the exudation of organic compounds by cyanobacteria under field conditions is available (Roger and Kulasooriya 1980).

Excretion of nutrients by aquatic plants is particularly pronounced in senescent plants and, undoubtedly, the largest proportion of nutrients tied up in plant tissues would be released after death (Kristritz 1978). Nutrients are released after death mainly because of microbial decomposition. However, Otsuki and Wetzel (1974) demonstrated under laboratory conditions that 30-40% of the net production of the submerged freshwater angiosperm *Scirpus subterminales* was released as dissolved OM on autolysis. Most of the autolytic OM was released within 5 d under both oxic and anoxic conditions.

A laboratory study by De Pinto and Verhoff (1977) illustrated the two mechanisms by which algae populations may decay under dark aerobic conditions—endogenous respiration by the algal cells themselves and decomposition by microorganisms. Active bacterial decomposition proved to be the more important mechanism by far. In the same study, the viability of the bacteria-free algal cultures after 70 d in the dark, with no net P regeneration, was regarded as indirect proof that bacteria not only can decompose algae but, under certain circumstances, can cause the termination of an algal bloom. However, whether the lytic bacteria act as pathogens (and thus are the primary cause of decline) or act as saprophytes (decomposing the dead algal material resulting from other primary processes) is still not clear (Fallon and Brock 1979).

#### 4.7.2. Susceptibility to decomposition

The decomposition rate of algae and aquatic plants depends on the environment, the species, and the physiological state of the plant.

The susceptibility to microbial decomposition of 14 algal species was assessed by Gunnison and Alexander (1975) in pond water and with inocula from several environments. Some of the algae were decomposed within short periods, but others withstood decomposition for more than 4 wk. Resistance to decomposition was not related to the production of toxins but was correlated with the relative biodegradabilities of specific components of the algal walls such as polyaromatic compounds.

The decomposition of four N<sub>2</sub>-fixing cyanobacteria at two different physiological stages by various soil bacteria was examined by Watanabe and Kiyohara (1960). Within 10 d of incubation with the most active strain (*Bacillus subtilis*), about 40% of the N from autolyzed cells and 5% of the N from fresh cells was converted to NH<sub>4</sub><sup>+</sup>.

#### 4.7.3. Regeneration of nutrients in floodwater

Experiments on regeneration of nutrients from algae and aquatic plants have not yet been conducted in situ in ricefields. They were mostly conducted either in the laboratory or in enclosures placed in situ in freshwater bodies. Therefore, the test samples were cut off from the circulation occurring under natural conditions, and the validity of the results are limited by this *enclosure effect* but they may still help in understanding what happens in the ricefield.

Studies in which field- or laboratory-grown algae were placed in the dark and the changes in N, or P, or both were monitored for various periods were summarized by Foree et al (1970). They reported three general stages of activity with nutrient regeneration.

1. The stage immediately after dark conditions—usually the first 24 h—during which either a release to or absorption from solution or a release followed by an absorption of nutrients took place.
2. A stationary stage over several days during which net nutrient regeneration was zero.
3. The stage in which active nutrient regeneration occurred with a net release of nutrients to the solution, lasting a few hundred days.

The N and P regeneration of algae in dark aerobic (44 strains) and dark anaerobic (21 strains) conditions was studied by the same authors (Foree et al 1970) for periods ranging from 40 to 360 d. In aerobic conditions, on the average 50% of the initial N and P was regenerated, but the extent of regeneration ranged from 0 to nearly 100%. In anaerobic conditions, the extent of N and P regeneration averaged 40 and 60%, respectively, with a range similar to that for aerobic decomposition.

The dark aerobic decomposition of batch unialgal cultures inoculated with a natural bacterial community was studied in detail by De Pinto and Verhoff (1977). The P regeneration values ranged from 31 to 95% (mean 74%), with

higher percentages of release associated with higher initial cellular P. The conversion of particulate organic N to  $\text{NH}_4^+$  ranged from 51 to 94% (mean 74%). The incubation periods required for stabilization of the system varied from 29 to 55 d, about one-third of which was bacterial lag time. The P regeneration followed a pattern that indicated three stages.

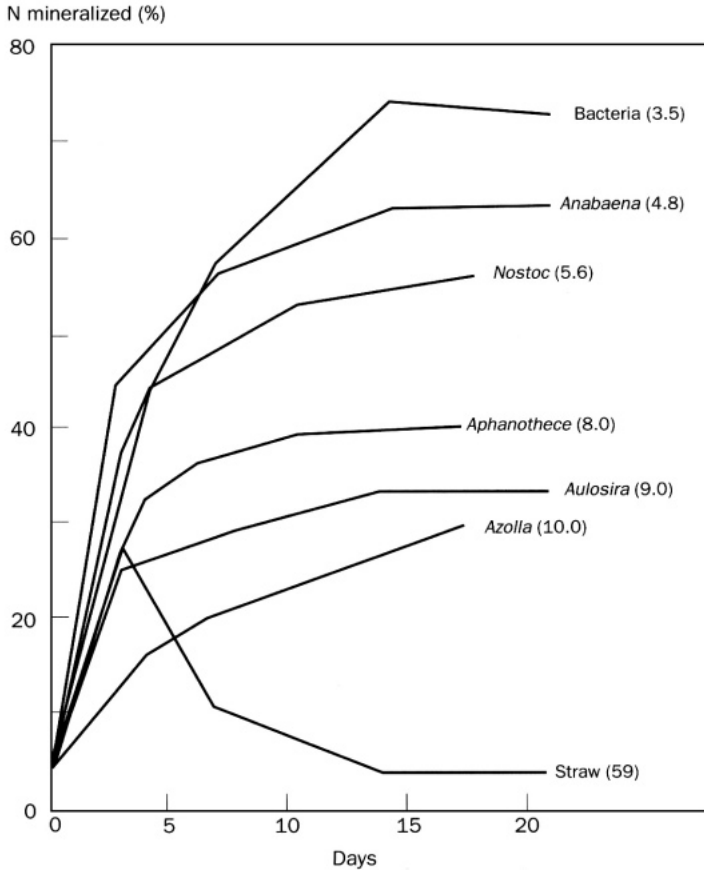
1. After the algae were subjected to a darkened environment, there was a rapid release of P to solution associated with endogenous respiration, followed by an immediate absorption by the remaining cells.
2. A stationary lag phase lasting several days, during which there was a buildup of bacteria and no net P regeneration.
3. When the viable algal population had been significantly reduced, there was an associated active P regeneration with a net release of orthophosphate to solution.

The regeneration pattern for N seemed to be less complicated than that for P. All organic N regenerated appeared first as  $\text{NH}_4^+$ ; then a portion of the  $\text{NH}_4^+$  was converted to  $\text{NO}_3^-$  by nitrification. The state of P regeneration during the active phase of decomposition (3d phase) depended on the initial P level, whereas N regeneration was a direct function of the amount of organic decomposition.

Studies dealing with the release of nutrients from decomposing aquatic macrophytes (Jewell 1971, Nichols and Keeney 1973, Kristritz 1978, Rho and Gunner 1978) have also dealt to different extents with the effects of the nutrients on the surrounding microflora, including algae. Kristritz (1978), who studied recycling of nutrients in an enclosed aquatic community of *Myriophyllum spicatum*, reported that total suspended bacterial biomass represented an average of 10% of the total organic N and P pool of the water column. Decaying *Myriophyllum heterophyllum* released  $\text{NH}_4^+$  and phosphate in concentrations sufficient to promote algal growth. The oxidation of  $\text{NH}_4^+$  by resident nitrifiers had a striking impact on microfloral succession. Nitrification was accompanied by a decrease in pH, and thereafter by a decline in the numbers of bacteria and protozoa. Subsequently, coincident with the accumulation of nitrite and nitrate, the numbers of the resident green algal communities rose dramatically.

#### 4.7.4. Mineralization in soil

Mineralization of some algae and weeds under flooded conditions was studied by Mitsui (1954). Nitrogen contents varied from 2.2 to 6.6%, C contents from 39 to 44%, and C-N ratios from 6.6 to 20.1. The order of the accumulation of  $\text{NH}_4^+$ -N followed the order of C-N ratios as long as the incubation period remained within 34 d. The largest quantity of  $\text{NH}_4^+$  was accumulated when *Lemna* (floating weed, C:N = 6.6) was incorporated whereas with *Spirogyra* (filamentous green alga, C:N = 20.1), accumulation was even less than in the control. A mineralization study by Roger et al (1986b) showed a highly significant negative correlation between the C-N ratio of the material and the



**4.9.** Nitrogen mineralization rates of cyanobacteria, *Azolla*, and straw. Numbers in parentheses are C-N values (Roger et al 1986).

percentage of N mineralized at a given time. With C-N ratios ranging from 3.5 to 10.0, between 30 and 65% of N from organisms found in PAB was mineralized in 3 wk (Fig. 4.9). The average C-N ratio of the various components of the photosynthetic biomass may range from 7 to 36 (Table 4.9).

A result contradictory to this C-N *rule* was cited by Rho and Gunner (1978): “Though Boyd (1974) reported a higher N content of phytoplankton tissue than that of macrophytes, the decomposition of macrophytes was found to be more complete and to occur at twice the rate of phytoplankton (Jewell 1971).”

The rate and degree of nitrification, under aerobic conditions, of different aquatic weeds added to the soil was studied by Riemer and Toth (1971) for 8 wk. Considerable variations were observed, with rates ranging from high values (40-60% for *Nuphar advena* and *Lemna minor* to low and even negative values (*Potamogeton pulcher*, *P. cordata*, and *Sparganium*). Some tissues not only showed poor nitrification but inhibited nitrification (old plants of *Phragmites*).

**Table 4.9. C:N of various components of algae and aquatic weeds (P.A. Roger, S.S. Ardales, and R.R. Jimenez, IRRI, 1989, unpubl.).**

	Data (no.)	Minimum	Maximum	Median
Cyanobacteria	400	4.0 <sup>a</sup>	10.8	6.7
<i>Azolla</i>	130	6.9	14.0	9.3
Submerged weeds	65	11.9	26.2	16.8
Broadleaf weeds	65	12.9	27.1	18.1
Grass weeds	63	26.4	55.0	35.9
Sedge weeds	61	14.5	30.9	22.1
All weeds	65	17.8	47.5	23.9

<sup>a</sup> Abnormally low value due to high (30.70%) ash/clay content in mucilaginous cyanobacteria.

#### 4.7.5. Effects on soil organic matter

Little is known about the nature of humus derived from algae and aquatic weeds. Their content of lignin, which is a major substance producing humus, seems to be low. Values ranging from 2.9% in *Hydrodictyon* (green alga) to 6.18% in *Hydrilla verticillata* (submerged weed) were recorded by Mitsui (1954), whereas ordinary green manures usually ranged from 9 to 24%.

Decomposition and humification of algal cells was studied by Verma and Martin (1976) using six strains of cyanobacteria and one green alga labeled with <sup>14</sup>C. After 22 wk incubation of the whole cell in a sandy loam, between 61 and 81 % of the added C had evolved as CO<sub>2</sub>. More than 50% of the residual <sup>14</sup>C activity in the soil was not extractable with 0.5% NaOH. Analysis of sedimentary OM from a cyanobacterial mat by Disnar and Trichet (1981) indicated a wealth of amino acids and carbohydrates and a paucity of aromatic structures. Dzumaniazou (1979) reported that green and blue-green algae stabilized humic acids and increased the content of humus and of free amino acids in an irrigated soil.

#### 4.7.6. Availability of N of the photosynthetic aquatic biomass to rice

Availability of algal N to rice has been quantified in <sup>15</sup>N experiments with cyanobacteria by Wilson et al (1980a), Tirol et al (1982), and Grant and Seegers (1985a) (Table 4. 10). Recovery of cyanobacterial N in the rice crop varied from 13 to 50%, depending on the nature of the algal material (fresh vs dried), the method of application (surface applied vs incorporated), and the presence or absence of soil fauna. Highest recovery (50%) was obtained when fresh material was incorporated in a soil depleted of fauna (Wilson et al 1980a). Lowest recovery was obtained when dried material was applied on the surface of a soil rich in tubificids (*Oligochaeta*) (Tirol et al 1982). Grant and Seegers (1985a) showed that tubificid activity reduced the recovery of algal N by rice by making the soil N available through a mineralization process.

Few data are available on the use by rice of N from macrophytes (Table 4.10). Shi et al (1980) reported that 25% of the N from incorporated <sup>15</sup>N-labeled

**Table 4.10. N of the photosynthetic biomass available to rice estimated from <sup>15</sup>N experiments (adapted from Roger et al 1987a).**

Material		N recovery (%)			Experiment type	Reference
Nature	State	Surface applied	Incorporated	Fauna <sup>a</sup>		
Cyanobacteria	Fresh	37	52	?	Pot	Wilson et al 1980a
Cyanobacteria	Dry	14	28	-	Pot	Tirol et al 1982
Cyanobacteria	Dry	23	23	+	Field	Tirol et al 1982
Cyanobacteria	Fresh	...	38	-	Pot	Tirol et al 1982
Cyanobacteria	Fresh	24	44	-	Pot	Grant and Seegers 1985a
Cyanobacteria	Fresh	25	30	+	Pot	Grant and Seegers 1985a
Cyanobacteria	Dry	...	35-40	-	Pot	Mian and Stewart 1985
Water hyacinth	Fresh	...	25	+	Field	Shi et al 1980
<i>Azolla pinnata</i>	Fresh	...	26	+	Field	Ito and Watanabe 1985
<i>A. caroliniana</i>	Fresh	12/14	26	+	Field	Ito and Watanabe 1985
<i>A. caroliniana</i>	Dry	...	34		Pot	Mian and Stewart 1985
<i>A. caroliniana</i>	Fresh	...	32	+	Field	Kumarasinghe et al 1986
<i>Azolla</i> sp.	Fresh	...	20	+	Field	Wen QiXiao et al 1987
Average		21	30			

<sup>a</sup> ? = unknown, - = absent, + = present.

water hyacinth was recovered in the rice crop. In a field experiment, Ito and Watanabe (1985) observed that when <sup>15</sup>N-labeled *Azolla* was placed at the surface of the soil, about 66% of *Azolla* N was lost and 12-14% was recovered in the rice plants. When *Azolla* was incorporated, losses were significantly reduced and availability increased to 26%.

The foregoing results indicate the following:

- Algae and aquatic weeds show great variation in their decomposition rate and in the conversion of plant N to NO<sub>3</sub><sup>-</sup> by soil microorganisms (Gunnison and Alexander 1975, Mitsui 1954, Riemer and Toth 1971).
- The extent of the decomposition of algae and aquatic weeds and the consequent regeneration of N and P into the water in a soluble form are similar in aerated and nonaerated conditions (Foree and McCarty 1970, Jewell and McCarty 1971, Rho and Gunner 1978).
- The relative regeneration rate of P from the algae (Golterman 1964) and from the macrophytes (Rho and Gunner 1978, Kristritz 1978) is much higher than that of N.

- Humus resulting from the decomposition of algae is poor in aromatic structures; because of their paucity in lignin, a similar characteristic for the humus from aquatic plants, especially submerged ones, can be expected.
- If the decomposition of the photosynthetic biomass occurs in the floodwater, the nutrient regeneration, along with many other parameters, can markedly affect the dynamic seasonal succession of the phytoplankton, but availability of the nutrients to the rice plant is poor.
- Nitrogen fixed or immobilized in the photosynthetic biomass is much more efficiently used by rice when incorporated into the soil.

#### 4.8. Algae and aquatic plants as weeds

Some of the aquatic macrophytes and algae developing in ricefields, and most of those developing in irrigation canals and water reservoirs connected to ricefields are considered as weeds. In a field, weeds compete with the crop for nutrients, space, and light. The importance of controlling weeds is emphasized in popularized books (see Vergara 1979).

Lists and identification keys for common aquatic weeds are provided in several handbooks (Smith et al 1977, Weldon et al 1979). A list of major rice weeds recorded in Asia was compiled by Moody (1981). Table 4.11 presents algal weeds and submerged and floating macrophytic weeds listed in Moody's compilation.

In ricefields, algae and submerged and floating plants are rarely considered weeds of major economic importance. Smith (1983) listed 74 plants considered as major rice weeds and classified them within three categories of decreasing importance (a,b,c). Among the 71 spp. listed in Table 4.11, only 3 (*Marsilea minuta*, *Salvinia molesta*, and *Spirodela polyrhiza* are classified in category b, and two (*Eichhornia crassipes*, and *Najas* sp.) in category c.

In a review on algal weeds and their chemical control, Das (1976) cited the genera *Chara*, *Spirogyra*, *Oscillatoria*, *Nitella*, *Oedogonium*, *Cladophora*, *Pitophora*, *Rhizoctonium*, and filamentous algae without specific names. It appears that only filamentous and higher algae are considered weeds while most macrophytes are. Batalla (1975) considers that most detrimental effects of algal weeds are observed during the first 30-40 d of the crop cycle in direct seeded rice (DSR) and during the first 2-3 wk of the crop cycle in transplanted rice.

The classification of a plant as a weed depends not only on the area but also on the method of crop cultivation. For example, submerged macrophytes such as *Chara* and *Hydrilla* are not often considered main weeds in transplanted rice (Moody 1981), but they are considered detrimental in DSR (Mukherji and Laha 1979).

**Table 4.11. Algae, and submerged and floating macrophytes listed as weed in Asia (adapted from Moody 1989).**

Weed		Type <sup>a</sup>	Country <sup>b</sup>
Genus	Species		
<i>Azolla</i>	<i>filiculoides</i>	F	Ido, Mal, Phi
	<i>pinnata</i>		Ban, Ido, Ind, Cam, Lao, Mal, Nep, Phi, Sri, Tha, Vie
	<i>rubra</i>		Ind, Tha
<i>Ceratophyllum</i>	<i>demersum</i>	S	Ban, Ido, Ind, Lao, Mal, Phi, Sri, Tha, Vie
<i>Chara</i>	<i>fibrosa</i>	S	Ind
	<i>globularis</i>		Ind
	<i>gymnopitis</i>		Mal
	sp.		Ban, Lao, Nep, Sri, Vie, Phi
	<i>vulgaris</i>		Phi
	<i>zeylanica</i>		Ind, Tha
<i>Chladophora</i>	sp.	A	Ind, Nep, Vie
<i>Eichhornia</i>	<i>azurea</i>	F	Ind, Phi
	<i>crassipes</i>		Ban, Ido, Ind, Cam, Lao, Mal, Nep, Phi, Sri, Tha, Vie
<i>Enteromorpha</i>	<i>intestinalis</i>	A	Vie
<i>Hydrilla</i>	<i>verticillata</i>	S	Ban, Bur, Ido, Ind, Cam, Lao, Mal, Nep, Pak, Phi, Sri, Tha, Vie
<i>Hydrocharis</i>	<i>dubia</i>	S	Ind, Nep, Tha
	<i>morsus-ranae</i>		Nep
<i>Hydrodictyon</i>	<i>reticulatum</i>	A	Ind
<i>Lemna</i>	<i>equinoctialis</i>	F	Ido, Ind, Mal, Phi, Tha
	<i>minor</i>		Ido, Ind, Mal, Nep, Pak, Tha, Vie
	<i>oligorhiza</i>		Ind
	<i>tenera</i>		Bur, Mal
	<i>trisulca</i>		Ban, Ind, Mal, Phi
<i>Marsilea</i>	<i>coromandeliana</i>	F	Ind
	<i>minuta</i>		Ban, Ido, Ind, Cam, Lao, Mal, Pak, Phi, Sri, Tha, Vie
			Ban, Bur, Ido, Ind, Cam, Lao, Mal, Nep, Pak, Phi, Sri, Tha, Vie
<i>Myriophyllum</i>	<i>quadrifolia</i>		Sri, Tha, Vie
	<i>alternifolium</i>	S	Ind
	<i>aquaticum</i>		Ido, Kam
	<i>indicum</i>		Ban, Ind, Vie
	<i>spicatum</i>		Ban, Ind, Vie
	<i>tetrandum</i>		Ind
	<i>tuberculatum</i>		Ban, Ind
<i>Najas</i>	<i>graminea</i>	S	Ban, Bur, Ido, Ind, Mal, Phi, Tha, Vie

**Table 4.11 continued.**

Weed		Type <sup>a</sup>	Country <sup>b</sup>
Genus	Species		
<i>Nechamandra</i>	<i>indica</i>		Ido, Ind, Cam, Phi, Vie
	<i>malesiana</i>		Ban, Bur, Ido, Ind, Mal, Phi, Vie
	<i>minor</i>		Ban, Ind
	<i>alternifolia</i>	S	Ban, Ind, Vie
<i>Nitella</i>	sp.	S	Ban, Ind, Tha, Vie
<i>Nostoc</i>	<i>carneum</i>	C	Phi
	<i>ellipso sporum</i>		Phi
<i>Oscillatoria</i>	sp.		Nep
	sp.	C	Ind
<i>Phormidium</i>	sp.	C	Ind
<i>Pistia</i>	<i>statiotes</i>	F	Ban, Ido, Ind, Cam. Lao, Mal, Nep, Phi, Sri, Tha, Vie
<i>Pithophora</i>	sp.	A	Ind
<i>Potamogeton</i>	<i>crispus</i>	S	Ban, Ind
	<i>distinctus</i>		Ind
	<i>miduhikimo</i>		Ind
	<i>mucronatus</i>		Tha
	<i>nodosus</i>		Ind
	<i>oblongus</i>		Tha
	<i>octandrus</i>		Ind, Pak
	<i>pectinatus</i>		Ind
	<i>perfoliatus</i>		Ind, Phi
	<i>perversus</i>		Tha
	<i>Salvinia</i>	sp.	
<i>cucullata</i>		F	Ido, Ind, Kam, Lao, Mal, Tha, Vie
<i>molesta</i>			Ido, Ind, Mal, Phi, Sri, Tha
<i>natans</i>			Ban, Ido, Ind, Mal,
<i>Spirodela</i>	sp.		Nep
	<i>polyrhiza</i>	F	Ido, Ind, Mal, Nep. Pak, Phi, Tha
<i>Spirogyra</i>	sp.		Vie
	<i>longata</i>	A	Ind
<i>Vallisneria</i>	sp.		Ban, Phi, Sri
	<i>spiralis</i>	S	Ban, Ind, Nep. Pak
<i>Vaucheria</i>	<i>verticillata</i>		Sri
	sp.	A	Ind
<i>Wolfia</i>	<i>arrhiza</i>	F	Ind, Mal, Phi
	<i>globosa</i>		Ind
	<i>microscopia</i>		Ind
	sp.		Nep, Vie

<sup>a</sup> A = algae, C = cyanobacteria, S = submerged weed, F = floating weed. <sup>b</sup> Ban = Bangladesh, Bur = Myanmar (Burma); Ido = Indonesia, Ind = India, Cam = Cambodia, Lao = Laos. Mal = Malaysia, Nep = Nepal, Pak = Pakistan, Phi = Philippines, Sri = Sri Lanka, Tha =Thailand, Vie = Vietnam.

#### 4.8.1. Unicellular and filamentous microalgae

Microalgae are usually not considered detrimental to transplanted rice except by favoring N losses by  $\text{NH}_3$  volatilization (see sect. 4.6.4).

However, in DSR, blooms of microalgae might have detrimental effects early in the crop. They are responsible for the so-called *slime* or *scum*. In India, scum problems were reported as early as 1928 (Vasistha 1928). In Australia, farmers recognize a brown slime dominated by diatoms, which partly develops epiphytically, and a green slime dominated by filamentous green algae developing either as floating mats or epiphytically (Noble and Happey-Wood 1987). Filamentous microalgae, especially those of the net-forming type, are detrimental at germination because of competition for light. Some mucilaginous epiphytic algae may cover young germinating roots and restrain them from penetrating the soil. Batalla (1975) reported a detrimental effect of *Oscillatoria* spp. when forming a dense membranaceous mat, especially in seedbeds, that restrains gaseous exchanges between soil and water and restrains root penetration into the soil. Microalgae may also have detrimental mechanical effects when their epiphytic growth either pulls seedlings down into the water or mud (Smith et al 1977), or lifts them up and uproots them when the water level varies.

$\text{N}_2$ -fixing cyanobacteria (*Nostoc*, *Anabaena*) have been sometimes cited as weeds in DSR because of their mechanical effect on seedlings (Smith et al 1977). However, the usefulness of these  $\text{N}_2$ -fixing microorganisms as a source of N in ricefields is well documented (Roger and Kulasoorya 1980).

#### 4.8.2. Macrophytic algae and submersed weeds

Most of the important submersed macrophytic weeds in freshwater bodies belong to the genera *Hydrilla*, *Myriophyllum*, *Ceratophyllum*, *Egeria*, *Elodea*, *Najas*, *Potamogeton*, *Vallisneria*, *Nitella*, and *Chara*. Macrophytic algae (*Chara*, *Nitella*) are not often considered detrimental because they usually develop late in the crop cycle, when the rice plant is tall enough not to suffer from mechanical disturbance or competition for light. However, when an early growth occurs, it may hamper the formation of tillers, restrain plant growth, and increase the number of empty spikelets (Mukherji and Sengupta 1964).

Reported detrimental effects of the lack of submerged weed control range from a slight decrease in yield to intensive damage. In ricefields in southern India, a biomass of 9-15 t *Chara* fw/ha caused yield losses of more than 30% (Misra et al 1976). In West Bengal, yield reductions of 10-20% were attributed to *Chara* and *Nitella* (Mukherji 1968). *Limnocharis* and *Eichhornia* invading ricefields in Sri Lanka were reported to take large areas out of production (Kotalawa 1976).

#### 4.8.3. Floating weeds

Among floating weeds, water hyacinths (*Eichhornia* spp.), *Salvinia* spp., water lettuce (*Pistia stratiotes*), and duckweeds (Lemnaceae family) are the most common.

Subsistence farmers in the wet lowlands of Bangladesh annually face disaster when rafts of water hyacinth, weighing up to 300 t/ha, float over their ricefields. As the floods recede, the weeds remain on the germinating rice and kill it (NAS 1976). Similar effects have been reported for *Salvinia* and *Pistia* (Varshney and Singh 1976, Kotalawa 1976).

*Azolla*, sometimes classified as a weed, has a potential as a biofertilizer (see sect. 7.3).

The PAB has two negative effects on the N balance. Submersed plants, by increasing the pH of submersion water, cause losses of N fertilizer by volatilization (Vlek and Craswell 1979). Weeds may also reduce photodependent BNF as shown by negative correlations observed between the N<sub>2</sub>-fixing cyanobacteria biomass and the submerged weed biomass (Roger and Kulasooriya 1980) or the floating weed biomass (Srinivasan 1982).

In the field, PAB can have both detrimental and beneficial effects. In irrigation canals and water reservoirs, however, it seems to have a mainly detrimental effect. It reduces the water flow in canals and the utility of reservoirs for water storage, irrigation, and fish production. Aquatic plants also increase water losses by transpiration from their leaves (National Science Research Council of Guyana 1973, Varshney and Singh 1976).

## 5 Fauna

After an extensive literature survey, Fernando et al (1979, 1980) concluded that the ricefield aquatic fauna spans the whole spectrum of freshwater fauna and may even include some brackish water species in river deltas, e.g., polychaetes and penaeid prawns. This high species diversity was attributed to a rapid recolonization of the ricefield by the fauna from contiguous water bodies, after disturbances caused by cultural practices or drying. The study by Heckman (1979) of a Thai ricefield for 1 yr also indicated wide faunal diversity with the record of 268 animal species. Rotifera (50 species) and Arthropoda (146 species) were the most numerous taxa, but 18 species of fish were also recorded. Recent studies indicate a decrease in species diversity under intensified cultivation (see sect. 6.1).

Methods for studying aquatic and soil fauna in wetland ricefields are presented in the appendix. This chapter surveys the components of the fauna, their dynamics during the crop cycle, and their major activities.

### 5.1. Components

The dominant groups of aquatic invertebrates are ostracods, copepods, cladocerans, rotifers, insect larvae, aquatic insects, molluscs, oligochaetes, and nematodes (Heckman 1979, Lim 1980, Roger and Kurihara 1988). They have both agricultural and environmental impacts as nutrient recyclers, rice pests, pest predators, and vectors of human and animal diseases.

The ricefield fauna directly responsible for the breakdown of the photosynthetic aquatic biomass (PAB) consists of microcrustaceans, insect larvae, and gastropods that graze on algae. These, together with the protozoans and rotifers, also recycle nutrients from decaying photosynthetic biomass. Translocation of primary production and its breakdown products from the surface to the deeper soil layer is expedited by aquatic oligochaetes, mostly tubificid worms (Grant and Seegers 1985b). Secondary and tertiary consumers (carnivorous insects and fishes) also recycle nutrients in the ecosystem or cause an exportation of nutrients when collected for food by farmers. Invertebrate predators that contribute to nutrient cycling and the control of pest outbreaks include species of Odonata, Coleoptera, and Notonectidae.

Few aquatic invertebrates are rice pests. An important aspect of ricefield fauna is the occurrence of vectors of human and animal diseases, mostly mosquitoes and snails. Wetland rice culture and irrigation schemes in tropical and subtropical regions create ecological conditions favorable to the propagation of vector-borne diseases (Roger and Bhuiyan, 1990). The most important are malaria, schistosomiasis, and Japanese encephalitis, whose vectors require an aquatic environment, either permanently or at certain stages of their life cycle (see sect. 5.5.2).

### 5.1.1. Macrofauna

Most studies on floodwater macrofauna deal with animals that constitute a possible source of food, with the emphasis on fish. Numerous fish species inhabit the ricefield ecosystem and are traditionally collected as food. The rice-associated food fish species were reviewed by Ruddle (1982). Rice-fish culture is presented in sect. 7.6.

### 5.1.2. Molluscs

Aquatic snails are very common inhabitants of ricefields where they can develop large populations, especially when organic manure is applied. Behavior experiments (Kurihara and Kadowaki 1988) showed that when snails had to choose between various soils, they were most often attracted (70-80%) to rice soils than to other soil types. The causative agent was a water-soluble substance.

Populations of small-size *Limnea* spp. up to 1,000 m<sup>2</sup> (1,500 kg/ha) have been observed in Philippine ricefields (Grant et al 1986). Large species (*Pila* spp., *Pomacea* spp., *Ampullaria* spp.) can develop populations of 1-10/m<sup>2</sup>. Some are detrimental to rice, grazing on seedlings (Saxena et al 1987). Farmers collect them for food or as feed for ducks, or simply remove them from the field and crush them on the dikes. Other species (*Bilinus* spp., *Biomphalaria* spp., *Limnea* spp.) are detrimental as vectors of bilharziosis (see sect. 5.6).

In experimental fields in the Philippines where large snails (*Pomacea canaliculata*) were removed as part of the management — a common farmer practice — *Melanoides tuberculata* and *M. granifera* were dominant, although *Stenomenalia fuscata* and *Limnea quadrasi* were recorded in all treatments and throughout the two seasons of the study (Simpson et al 1994c). Maximum populations after transplanting reached 1,300 animals/m<sup>2</sup> (690 kg/ha) in fallow plots, and 660 animals/m<sup>2</sup> (380 kg/ha) in planted nonfertilized plots. Populations were lower in fertilized plots (Table 5.1). A similar observation was made by Kurihara et al (1987) who observed a productivity of snails 4-5 times larger in nonplanted plots (7-15 g flesh dry weight [dw]/m<sup>2</sup> after 4 mo) compared with planted plots (3.2-3.5 g flesh dw/m<sup>2</sup>). Aquatic snails prefer habitats devoid of higher plants but with 1) a rich microbial and microalgal flora, or 2) decaying plant material, which provides their principal food (Webbe 1988). In experiments at IRRI (So et al 1989), straw applied in the floodwater

**Table 5.1. Population density and biomass estimates of molluscs in ricefields after transplanting (Simpson et al 1994c).<sup>a</sup>**

Treatment	Densities (no./m <sup>2</sup> )			Biomass (fresh wt/m <sup>2</sup> )		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Planted, no fertilizer	0	981	206	0	680	140
110 kg N/ha	0	341	38	0	300	50
110 kg N/ha + pesticides <sup>b</sup>	0	405	75	0	370	90
<i>Azolla</i> <sup>c</sup>	16	581	156	5	290	130
Fallow	105	1307	352	60	690	190

<sup>a</sup>Average of measurements for two crop cycle in five replicated plots. <sup>b</sup>One application of 0.3 kg al/ha butachlor and five applications of 0.5 kg ai/ha of carbofuran. <sup>c</sup>Grown in the field and incorporated before transplanting.

2 wk before incorporation and rice transplanting markedly increased populations of snails, which were almost absent in the control plots.

Although snails may not prefer any particular species of microflora, the quantitative composition of their diet is probably important in conditioning their habitat. *Oncomelania quadrasi* was positively correlated with the presence of green algae, but negatively with cyanobacteria (Webbe 1988).

Aquatic snails can multiply rapidly. *Oncomelania hupensis* can live up to 200 d; other species reportedly live longer. *Oncomelania quadrasi* starts laying eggs by the age of 90-100 d or by the time it reaches 3.5 mm. A single copulation enables a snail to lay eggs for several days. An average of 2 eggs are laid every 5 d. With an egg survival rate of 16%, each adult snail could produce an average of 4.4 adults in the next generation (Garcia 1988).

### 5.1.3. Zooplankton

Zooplankton and benthic invertebrates have almost exclusively been quantified in fertilized fields and the units of density are not usually comparable (Table 5.2). In Japan, Kurasawa (1956) recorded 198 *Daphnia*, 15 *Bosmina*, 42 *Cyclops*, 42 *Branchionus*, and 56 *Keratella* /liter about 6 wk after planting. Crustacean zooplankton densities ranged from 200 to 800/liter in another Japanese study (Kikuchi et al 1975). Large populations of ostracods ( $10 \times 10^3$ - $20 \times 10^3$ /m<sup>2</sup>) and chironomid larvae (8,000/m<sup>2</sup>) have been observed in Philippine ricefields (Grant et al 1986). A survey of aquatic invertebrates in experimental ricefields in the Philippines subjected to a range of fertilizer and pesticide treatments showed that ostracods, copepods, cladocerans, mosquito, and chironomid larvae can develop significant population densities (Simpson et al 1994b) (Table 5.2). Among those, ostracods were the most abundant, reaching densities higher than  $30 \times 10^3$ /m<sup>2</sup>. Their abundance was enhanced by N fertilizer broadcast in floodwater because it favored the growth of unicellular algae on which they feed.

**Table 5.2. Population density estimates of crustacean zooplankton and dipteran larvae in ricefields.**

Organisms and density <sup>a</sup>	Country	Reference
Crustacean zooplankton		
200-800/liter	Japan	Kikuchi et al 1975
0-50,000/m <sup>2</sup>	Japan	Kurasawa 1956
Cladocerans		
max. 300/liter	Malaysia	Ali 1990
0-33,000 (900)/m <sup>2</sup>	Philippines	Simpson et al 1994b
0-1100 (30)/liter	Philippines	Simpson et al 1994b
<i>Daphnia</i> 198/liter	Japan	Kurasawa 1956
<i>Bosmina</i> 15/liter	Japan	Kurasawa 1956
Copepods		
max. 800/liter	Malaysia	Ali 1990
0-40 000 (6,000)/m <sup>2</sup>	Philippines	Simpson et al 1994b
0-1700 (180)/liter	Philippines	Simpson et al 1994b
<i>Cyclops</i> 42/liter	Japan	Kurasawa 1956
Ostracods		
300-2,400/liter	Philippines	Lapis, in Simpson et al 1994b
0-422/liter	Philippines	Grant et al 1983a
10-20,000/m <sup>2</sup>	Philippines	Grant et al 1986
300-37,000/m <sup>2</sup>	Malaysia	Lim and Wong 1986
0-98,000 (6,000)/m <sup>2</sup>	Philippines	Simpson et al 1994b
0-4300 (200)/liter	Philippines	Simpson et al 1994b
Dipteran larvae		
Chironomid larvae		
0-22,000/m <sup>2</sup>	Philippines	Lapis, in Simpson et al 1994b
8000/m <sup>2</sup>	Philippines	Grant et al 1986a
0-10,000 (600)/m <sup>2</sup>	Philippines	Simpson et al 1994b
0-700 (20)/liter	Philippines	Simpson et al 1994b
Mosquito larvae		
0-7000 (170)/m <sup>2</sup>	Philippines	Simpson et al 1994b
0-350 (10)/liter	Philippines	Simpson et al 1994b

<sup>a</sup> Median values are indicated in parentheses.

Ostracods seem to be ubiquitous in ricefields. Species originating from Africa, Asia, South America, and Australia were recorded in Italian ricefields (Fox 1965, Ghetti 1973). Ostracods from ricefields of Southeast Asia have been studied by Victor and Fernando (1980), who found them in about half of the ricefield samples and recorded a number of common and widely distributed species.

Copepods and cladocerans, though usually recorded in ricefield floodwater, have not yet been reported to have become the dominant component of the zooplankton in fertilized ricefields.

#### 5.1.4. Insects

Most of the information on ricefield insects deals with insect pests developing in the rice canopy. A list of aquatic and semiaquatic heteroptera from ricefields of the Philippines was compiled by Yano et al (1981). Many secondary insect consumers in floodwater belong to this order and to the Odonates. In terms of floodwater ecology, insect larvae that are often dominant include those of chironomids and mosquitoes.

Chironomids are very common inhabitants of ricefields. Populations of larvae up to  $1.8 \times 10^4/m^2$ , corresponding to 8 species and 7 genera, were recorded in California ricefields (Clement et al 1977). Chironomids were reported to be the most numerous insects in the ricefields of the Republic of Korea. Their larvae feed on microalgae; in turn, predators such as spiders and damselflies feed on them. They serve as an alternative food of predators and thus help to conserve predators of rice pests when those are scarce (Yatsumatsu et al 1979).

Mosquitoes are ubiquitous in ricefields. Their reproduction and abundance are affected by plant height, water depth, soil, other environmental conditions, and cultural practices.

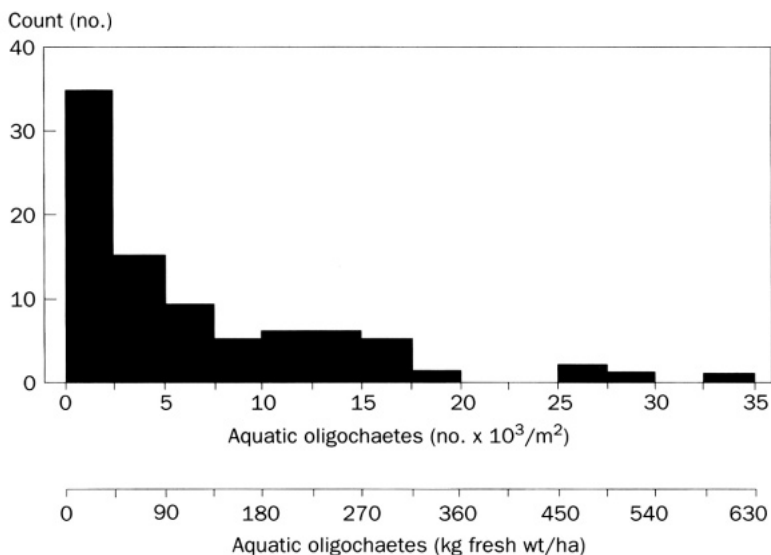
Mosquito production in ricefields ranges from about 2/m<sup>2</sup> per day for *Anopheles* (Hill and Cambournac 1941) to about 20/m<sup>2</sup> per day for *Culex tritaeniorhynchus* (Heathcote 1970). Larvae populations of up to 3,500/m<sup>2</sup> were recorded in the Philippines (Simpson et al 1994b).

A succession of species was observed in ricefields of Burkina Faso (Carnevale and Robert 1987). Heliophilic species were prevalent during early stages of rice growth, but were gradually replaced by more shade-loving species with the increase in plant height and canopy development. *Anopheles gambiae* developed from soil flooding to the booting stage of rice. From booting to heading, *A. pharoensis* was dominant; it was then replaced by *A. coustani* during the ripening phase.

Some sun-loving species are found mainly in waters without vegetation, whereas many others prefer the presence of vegetation. Vegetation is necessary for *Mansonia* because both larvae and pupae obtain their O<sub>2</sub> requirements by piercing submerged roots or stems. Wherever water lettuce (*Pistia*), water hyacinth (*Eichhornia*), or *Salvinia* are found, breeding by *Mansonia* should be suspected, although this species can use other aquatic plants (Mather and Trinh 1984).

#### 5.1.5. Benthic invertebrates

Oligochaetes, especially tubificidae, are an important component of the benthic fauna ensuring nutrient exchange between the soil and the floodwater. Tubificid population of about  $10^4/m^2$  were recorded in IRRI fields (IRRI 1985). In Japan, tubificids (*Branchiura sowerbyi*, *Limnodrilus socialis*) increased from a few at the beginning of the crop cycle to more than  $4 \times 10^4/m^2$  at the end of



**5.1.** Histogram of counts of oligochaetes in 32 farmers' fields at 100 d after transplanting (DT) (1989 dry season), and 4 and 90 DT (1989 wet season), and estimated corresponding biomass (Simpson et al 1994b).

the crop in a field where organic matter (OM) was incorporated (Kikuchi et al 1975).

Surveys of oligochaete populations in experimental plots at the IRRI farm and 32 farmers' fields of Laguna Province, Philippines, showed that populations were dominated by *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi*, were contagiously distributed, and ranged from 0 to  $4 \times 10^4/m^2$  (Simpson et al 1993a,b). Estimates of biomasses ranged from 0 to 630 kg fw/ha (av about 100 kg/ha) (Fig. 5.1). Average populations at each site were positively correlated with total soil organic C and the quantity of N fertilizer applied. This relationship was probably mediated through bacterial decomposers used by the oligochaetes as a food, and agree with the positive effect of OM incorporation on tubificid populations observed at the IRRI farm (IRRI 1990) and in Japan (Kikuchi et al 1975). Similar relationships have been shown for oligochaetes in nonflooded soils.

A positive correlation was observed between tubificid populations and the quantity of fertilizer applied (Simpson et al 1993a,b). It presumably resulted from an increased aquatic primary production, which provided food for oligochaetes. The importance of primary production for aquatic oligochaetes was indirectly demonstrated by the absence of, or the extremely low, populations in microplots where photosynthetic activity in the floodwater was restricted (Simpson et al 1993a). Populations were also correlated negatively with soil bulk density and positively with soil moisture at sampling

time. The positive correlation with soil moisture probably reflected respiratory stress and desiccation. In drying soils, no tubificids were usually recorded in the first 10 cm of soil (Simpson et al 1993a,b).

Saprophytic nematodes probably play a significant role in recycling nutrients. Microscopic observations by the author showed that they are often abundant in algal blooms and in the detritus layer at the soil-water interface. However, little quantitative data is available on these organisms. In Japan, Ishibaschi and Itoh (1981) recorded populations of saprophytic nematodes ( $10^6/m^2$ ), about one-hundred times larger than those of parasitic nematodes.

## 5.2. Dynamics of invertebrate populations during the crop cycle

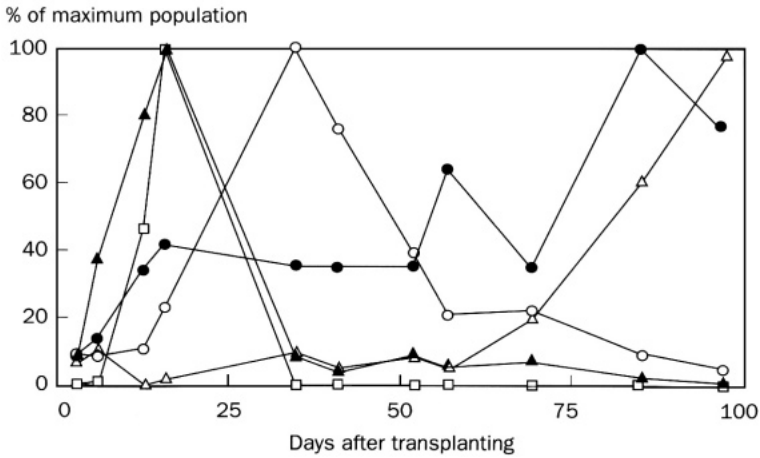
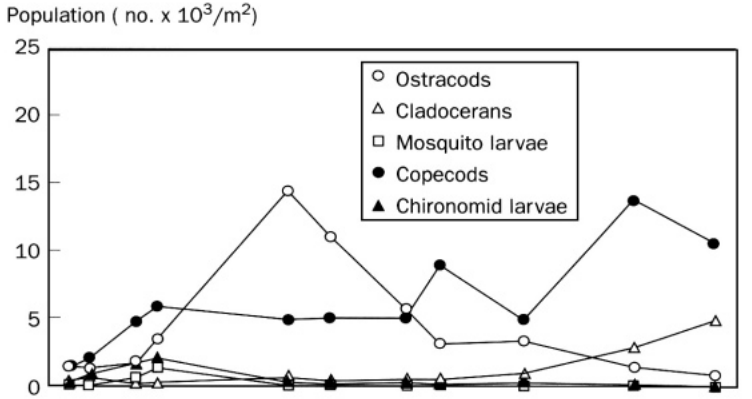
Records of the dynamics of the floodwater biota during a crop cycle and under a range of agricultural practices are scarce, and deal mostly with zooplankton of temperate ricefields (Kurasawa 1956, Kikuchi et al 1975, Moroni 1967, Pont 1978, Rossi et al 1974, Ferrari 1984). Detailed data for tropical ricefields are available in Simpson et al (1993a,b; 1994a,b,c) who studied the effects of N fertilizer, pesticides, and green manure application on the floodwater ecology of a tropical ricefield. They monitored the dynamics of 1) floodwater primary producers, and 2) populations of ostracods, copepods, cladocerans, chironomid and mosquito larvae, and aquatic molluscs for the duration of a crop cycle.

The dynamics of aquatic molluscs are strongly influenced by the fallow period preceding the rice crop. Under wet fallow, populations increase but dry fallow decreases their incidence. In planted fields, aquatic mollusc populations, expressed either numerically or as biomass, usually declined as the crop season progressed. That was attributed to a decrease in availability of suitable food, partly due to competition by other fauna (Simpson et al 1994c).

Aquatic invertebrate populations recorded by Simpson et al (1994b) in the Philippines were successional: mosquito and chironomid larvae > ostracods > copepods, cladocerans (Fig. 5.2). The early development of mosquito and chironomid larvae, rapidly succeeded by ostracods, was attributed to the blooming of phytoplankton after fertilizer application, because these organisms are algal grazers. Their proliferation was not observed in plots where algal growth was poor because N fertilizer was either not applied or was deep-placed (see also Fig. 6.1).

In Japan, plots subjected to a variety of weed and organic manure management practices consistently showed a peak of zooplankton early in the crop cycle, but in fields where organic manure had been incorporated before the rice crop, a second peak of zooplankton was observed later in the cropping season.

Mosquito larvae are generally low after transplanting, peak a few weeks later, and then decline as the plants reach a height of 60-100 cm. Besides the effect of fertilizer management, that was also attributed to physical obstruc-



**5.2.** Average dynamics of zooplankton populations during a crop cycle in the Philippines. Each value is the average of 13 treatments with 5 replications (Simpson et al 1994b).

tion of oviposition, increased shade, and the establishment of predators in the fields (Goonasekere and Amerasinghe 1988). Mosquito reproduction normally ceases when the fields are drained before harvest, but may continue at a low level in residual pools (Goonasekere and Amerasinghe 1988).

Data available on aquatic oligochaetes are scarce and do not show general trends. Crop cycle dynamics reported in Japan (Kikuchi et al 1975) roughly showed populations that remained low during the first half of the crop cycle and increased during the second part of the cycle. Dynamics reported in the Philippines by Simpson et al (1993a) were more complex, exhibiting one or two peaks and a maximum population density occurring earlier than in Japan, at 30 or 50 d after transplanting (DT). In a survey of 32 farms, the com-

parison of oligochaete densities at the beginning and the end of the crop cycle showed that populations could exhibit any behavior—increasing, decreasing, or remaining constant (Simpson et al 1993b).

Data are also available for ciliate in an Italian ricefield. Their population fluctuated between  $10^3$  and  $10^5$ /liter during most of the crop cycle and declined during the last month. Their total production was estimated to be 471 KJ/m<sup>2</sup>, while energy losses due to respiration accounted for 178 KJ/m<sup>2</sup> (Madoni 1987).

### 5.3. Grazing

#### 5.3.1. Nature of grazers

Animals that obtain all or part of their food by filtering, browsing, or shredding primary production are called grazers. Planktonic grazers, such as copepods, cladocerans, and rotifers filter phytoplankton and bacteria from the floodwater. At the soil-water interface, ostracods, chironomid larvae, mosquito larvae, and molluscs browse epipellic algae. Ostracods, cladocerans, and copepods are microcrustaceans that are widespread in ricefields, apparently as a result of rice cultivation (Fernando 1977). Molluscs and aerially dispersed chironomids are also widespread grazers, except in low pH environments such as acid-saline, acid-sulfate, and Fe-toxic soils. Generally, species diversity in freshwater ecosystems is positively correlated with increasing pH (Townsend et al 1983), but other physical and chemical factors such as temperature, water movement, desiccation, O<sub>2</sub>, and Ca also affect the range and distribution of invertebrate species (Grant et al 1983a).

Studies on grazing in ricefields were conducted after zooplankton was identified as a major limiting factor for cyanobacteria growth and a cause of failure of algal inoculation in ricefields (Watanabe et al 1955, Hirano et al 1955), and after the use of insecticides to control rice pests was seen to increase algal growth (Raghu and MacRae 1967). These authors implicated the cladocerans, copepods, and ostracods as detrimental to cyanobacteria establishment. Larger invertebrates, including chironomid larvae and snails, are also heavy grazers, not only of epipellic and floating algae but, in the case of pulmonate molluscs, of algae epiphytic on rice stems.

#### 5.3.2. Susceptibility of algae to grazing

In laboratory studies, zooplankton grazing on algae (mostly cyanobacteria) exhibit diet preferences (Wilson et al 1980b, Grant and Alexander 1981, Osa-Afiana and Alexander 1981). Preferences were related to the ability of the strain to support grazer growth and reproduction in culture (Grant and Alexander 1981, Osa-Afiana and Alexander 1981). Arnold (1971) observed that ingestion, assimilation, survivorship, and reproduction of *Daphnia pulex* was better when fed on green algae (*Chlorella*, *Ankistrodesmus*) than on cyanobacteria (*Anacystis*, *Synechococcus*, *Synechocystis*, *Anabaena flos-aquae*, *Gloeocapsa*). Three

of the cyanobacteria tested showed some toxicity and inhibition toward *D. pulex* and none of the strains was considered able to provide sufficient nutrition to support a population that does not have other food available (Arnold 1971). On the other hand, Foote (1981) reported that larvae of *Pelina truncatula* fed preferentially on cyanobacteria and were able to complete their development on some genera (*Cylindrospermum*, *Anabaena*, *Lyngbia*, *Oscillatoria*).

Some strains appear to be resistant to specific grazers (Arnold 1971), but this probably does not protect them against the whole spectrum of grazers, which might be wide. In a survey of 12 ricefields in the Philippines, 68% of the recorded taxa of invertebrates were potential grazers (IRRI 1985). Little is known about the attributes that determine resistance to grazing. Toxicity is considered to be an effective defense mechanism in *Microcystis* (Lampert 1981). A general trend among N<sub>2</sub>-fixing cyanobacteria is that strains that form mucilaginous colonies (*Aphanothece*, *Nostoc*, *Gloeotrichia*) are less susceptible to grazing than strains that do not (Grant et al 1985). Their mucilage protects such strains against ostracods and molluscs, but not against chironomid larvae (Martinez et al 1977).

The size of the cells or filaments and their age might also influence the susceptibility to grazing. Wilson et al (1980b) observed that the growth of *Tolypothrix tenuis*, *Anabaena* sp., and *Aulosira* sp., estimated through acetylene-reducing activity, was strongly reduced after 3 d exposure to the cladoceran *Daphnia magna*, but the grazer had little effect upon the maximum development and growth rate of the algae. They suggested that short filaments of the inoculum were susceptible to grazing while longer filaments that developed thereafter were resistant. Young cultures of *Anabaena* and *Calothrix* were more readily ingested by *Cypris* sp. than old ones (Grant and Alexander 1981). Inasmuch as cell dimensions were similar and length of the filament did not affect grazing—as ostracods shred them—possibly some toxic or antifeeding compound was produced in older cultures.

Some information is available on grazing by gastropods. Feeding experiments on *Cipangopaludina japonica* (mud snail) (Kurihara and Kadowaki 1988) with *Spirogyra* sp., a filamentous green alga common in ricefields, showed that snails prefer to feed on partly decomposed algae rather than on fresh material. That was explained by a feeding preference for bacteria that develop on decomposing material. Comparison of various nutrient sources indicated that snails mainly use proteins, but not high-molecular weight carbohydrates. Depending on the nature of the substrate and the size of the animal, consumption rates ranged from 4 to 250 mg/d per g dw of animal without shell. Largest ingestion and assimilation rates were observed with young animals feeding on casein. Cellulose- or acetone-treated *Spirogyra* was not assimilated. However, the observation that snails kept on soil in the dark were losing weight and dying within few weeks showed their dependence on algal activity for obtaining food. The authors indicated the snails possibly feed on

algal excreta and graze on cyanobacteria.

### 5.3.3. Grazing rate

Quantitative data on grazing rate are limited but suggest a very significant activity of zooplankton. Grazing rates of ostracods on monospecific cultures of  $^{14}\text{C}$ -labeled cyanobacteria varied from 1 to more than 100  $\mu\text{g dw}$  alga/ostracod per day (Grant and Alexander 1981, Grant et al 1983b). Animal size and floodwater temperature significantly affected grazing rates, but neither light nor pH (in a range of 5-10) had much effect. Ingestion and excretion rates of *Heterocypris luzonensis* (Ostracoda) determined in the laboratory by Grant and converted to cyanobacteria consumed by a field population (8,700/m<sup>2</sup>) totaled 187 g N/ha per day, 118 g of which was excreted as  $\text{NH}_3$  (Roger et al 1987a). Field consumption extrapolated from these values is 73 kg fw alga/ha per day or 19 kg N/ha per crop, using 2.5% N on dw basis and assuming 1) a continuous supply of cyanobacteria, and 2) that the feeding behavior of ostracods does not vary. However, because environmental factors such as temperature,  $\text{O}_2$  concentration, food availability, turbidity, and cultural practices will modify grazing rates, estimates made under laboratory conditions can be used only with reservation (Grant et al 1986).

### 5.3.4. Grazing and biological $\text{N}_2$ fixation

The role of some invertebrate grazers in restricting  $\text{N}_2$ -fixing activities of cyanobacteria has been evaluated in laboratory experiments (Wilson et al 1980b, Osa-Afiana and Alexander 1981) and in situ (Grant et al 1985). These results collectively support the notion that primary consumers that proliferate at the expense of cyanobacteria are a major limiting factor to algal  $\text{N}_2$  fixation in lowland ricefields. Grazing decreases  $\text{N}_2$  fixation due to cyanobacteria by reducing their biomass, and it is obvious that grazer density during an algal bloom will affect the amount of  $\text{N}_2$  fixed. Grazer diversity is also important as negative correlations exist between the number of grazer taxa and both species diversity and abundance of cyanobacteria in rice soils (IRRI 1985). Specific  $\text{N}_2$  fixation of cyanobacteria, measured per unit biomass, might also be diminished by grazing (Osa-Afiana and Alexander 1981). In addition, the mechanical action of grazers at the soil surface increases water turbidity and decreases light available to photodependent  $\text{N}_2$ -fixing organisms.

Because of the relative higher resistance to grazing of cyanobacteria that form mucilaginous colonies, grazing has a selective effect on cyanobacteria flora. Plate counts showed that genera forming mucilaginous colonies were dominant in more than 90% of 102 soils studied (Roger et al 1987a). That indicates that grazing leads to the dominance of mucilaginous cyanobacteria, which are usually less active in biological  $\text{N}_2$  fixation (Antarikanonda and Lorenzen 1982, Grant et al 1985).

### 5.3.5. Grazing and nutrient cycling

Nutrient availability in the floodwater, especially N and P, determines algal growth and photodependent N<sub>2</sub> fixation. Nutrient recycling in the floodwater is performed by microorganisms, zooplankton, benthos, and certain invertebrate fauna (e.g., oligochaetes, chironomid larvae). Quantitative data on nutrient cycling through grazing in ricefield are lacking.

Excretion of inorganic and organic forms of N and P has been measured in lake microcrustaceans (Ganf and Blazka 1974, Gardner and Miller 1981, Smith 1978), protozoa (Sherr et al 1983), tubificids, and chironomids (Gardner et al 1983) and gastropods (Chatuverdi and Agarwal 1983). Ammonium N is generally the excretion product, but small amounts of urea are sometimes detected. Soluble reactive P, being the form most available to algae, is usually measured.

P excretion rate was related to crustacean dw by the equation

$$\mu\text{g P / specimen per d} = 1.047^{0.801w}$$

where w is weight expressed in mg (Gutel'makher 1983). Average P excretion expressed in nmol P/(mg ash-free dw) per h was 0.54 for *Chironomus* sp. and 0.17 for *Limnodrilus* sp. (Nalepa et al 1983). These rates, determined from animals living in lake sediments, reflect the rate of digestion of detrital particles. Release rates from the surface soil of ricefields are expected to be higher, as more readily digestible OM is available, especially at times of algal blooms.

Excretion rates of N measured under laboratory conditions and extrapolation to the field are presented in Tables 5.3 and 5.4. An excretion rate of 118 g NH<sub>3</sub>/ha per day by *Heterocypris luzonensis* was extrapolated from labo-

**Table 5.3. Excretion rates ( $\mu\text{g NH}_4^+$ /mg dry weight animal per day) of some grazers and extrapolated daily values (g NH<sub>4</sub><sup>+</sup>/ha per day) (Roger and Kurihara 1991).**

Organism	Excretion rate		Reference
	$\mu\text{g NH}_4^+$ / animal per day	g NH <sub>4</sub> <sup>+</sup> /ha per day	
<i>Limnea viridis</i>	... <sup>a</sup>	120-130	Roger et al 1987a
<i>Heterocypris luzonensis</i>	...	118	Roger et al 1987a
<i>Chironomus</i> sp.	6.3	25 <sup>c</sup>	Gardner et al 1983
<i>Limnodrilus</i> sp.	4.6 <sup>b</sup>	18 <sup>c</sup>	Gardner et al 1983
<i>Daphnia pulex</i>	5.4 <sup>b</sup>	22 <sup>c</sup>	Jacobsen and Comita 1976
<i>Thermocyclops hyalensis</i>	44.6 <sup>b</sup>	178 <sup>c</sup>	Ganf and Blazka 1974
<i>Heterocypris luzonensis</i>	42.3	169 <sup>c</sup>	I.F. Grant, NRI, 1986, pers. commun.

<sup>a</sup>... = not available. <sup>b</sup> Determined from animals living in lake sediment. <sup>c</sup>Extrapolated for a population of 40 kg fresh wt/ha.

**Table 5.4. Algal N recycled by ostracods (extrapolated from data of Roger et al 1987a and Osa-Afiana and Alexander 1981).**

Population	Zooplankton biomass <sup>a</sup>	Daily consumption		Daily N excretion
		Algal dw	Algal N	
1 animal	0.3 mg dw	43 µg/animal	1.7 µg/animal	1 µg/animal
4000/m <sup>2</sup>	12 kg fw/ha	1.4 kg dw/ha	68 g/ha	41 g/ha
20,000/m <sup>2</sup>	60 kg fw/ha	6.8 kg dw/ha	340 g/ha	205 g/ha
50,000/m <sup>2</sup>	150 kg fw/ha	17 kg dw/ha	850 g/ha	512 g/ha

<sup>a</sup>dw = dry weight, fw =fresh weight.

ratory measurements (Roger et al 1987a). Regeneration rates cannot be estimated until the dynamics of grazer populations and their diets have been quantified. With regard to the large grazer populations that can develop in ricefields, however, nutrient recycling by grazing is most probably a major factor for PAB productivity. Rapid algal successions, frequent at the beginning of the crop, may indicate a rapid turnover rate of the N and P pools.

#### 5.4. Aquatic oligochaetes and interactions between floodwater and soil

In flooded rice soils, the reduced soil has an important role in providing nutrients to the floodwater community. Mechanical disturbances resulting from cultural practices (especially weeding), heavy rains, and actions of the fauna break the interface between floodwater and soil and increase exchanges between the two environments. Among biotic factors, the benthic tubificids (oligochaetes) have received special attention because they can move back and forth between the reduced soil and the floodwater. They usually are concentrated in the upper layer of the soil where they displace soil and water by their burrowing and passage through their gut. Fry (1982) reviewed their role in aquatic sediments and Kurihara (1983) studied their role in flooded rice soils.

Information on the role of tubificids in wetland soils comes from 1) extensive in vitro experiments in 300-ml beakers (Kikuchi and Kurihara 1977,1982; Fukuhara et al 1980; Kikuchi et al 1977), 2) experiments in unplanted microplots (0.5 m<sup>2</sup>) with 1,000 animals/m<sup>2</sup> (Kikuchi and Kurihara 1982), and 3) field experiments (Kurihara and Kikuchi 1988). Tubificids were shown to affect weed growth, soil physicochemical and microbiological properties, and the nutritional status of floodwater and its flora and fauna.

#### 5.4.1. Effect on rooted weeds and soil particle distribution

Field observations of lower weed density in plots where tubificids were abundant (Kikuchi et al 1975) were confirmed by in vitro experiments showing that the addition of tubificids markedly decreased weed populations (Kikuchi and Kurihara 1977). *Monochoria vaginalis* was not much affected while other weeds, among which *Cyperus difformis* was dominant, were drastically reduced (Kikuchi et al 1977).

The same experiments showed that the soil oxidized layer observed in the controls disappeared in the beakers with tubificids because tubificids caused a vertical redistribution of soil particles (Kikuchi and Kurihara 1977). Larger particles and plant residues were gradually concentrated in the lower soil layer and finer particles in the upper layer. This redistribution explains the weeding effect of tubificids, which move seeds 3-5 cm under the soil surface (Kurihara 1983) into a zone where O<sub>2</sub> concentration is too low for their germination.

#### 5.4.2. Effect on soil and water chemistry

In the presence of tubificids, soil pH was 0.5-2.0 units higher, Eh was 0.05-0.15 V lower, and biological and nonbiological O<sub>2</sub> uptake of the soil was higher than in their absence. Lower Eh and higher O<sub>2</sub> uptake were attributed to the lower weed density in the presence of tubificids and a resulting lower oxidation of the soil because of less root biomass. That was confirmed by the absence of differences between soils with and without tubificids when samples were maintained in the dark and no weed could grow (Kikuchi et al 1977).

A higher concentration of Fe<sup>++</sup> and NH<sub>4</sub><sup>+</sup> in the soil in the presence of tubificids was mostly attributed to the decrease of weed populations (Kikuchi et al 1977, Kikuchi and Kurihara 1977). Differences were also recorded in dark incubation, however, indicating some direct effect of tubificids (Fukuhara et al 1980). Higher concentrations of acid-soluble particulate Fe, NH<sub>4</sub><sup>+</sup>, and hexose in the floodwater directly resulted from the tubificid action (Kikuchi and Kurihara 1977, Fukuhara et al 1980).

Microplot experiments (Kikuchi and Kurihara 1982) showed that the addition of 1,000 animals/m<sup>2</sup> to the soil increased the concentration of acid-soluble Fe, soluble phosphate, and NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> in the floodwater. Whereas tubificids increased NH<sub>4</sub><sup>+</sup> concentration in the floodwater in the dark (Kikuchi and Kurihara 1977), no increase in NH<sub>4</sub><sup>+</sup> was detected in outdoor microplots. This was attributed to the absorption of NH<sub>4</sub><sup>+</sup> by algae as indicated by a much larger algal biomass in the presence of tubificids (Kikuchi and Kurihara 1982).

#### 5.4.3. Effect on microflora

Tubificids increased the populations of soil anaerobes (sulfate-reducing bacteria) and decreased that of aerobes. That was observed both under a dark-light cycle and continuous dark, indicating that tubificids were directly responsible for the change (Kikuchi et al 1977, Kikuchi and Kurihara 1977). Ex-

periments in the dark showed higher populations of sulfate-reducing bacteria in the feces of the tubificids than in the soil, while total aerobic and anaerobic populations were similar. A reduction of  $\text{NO}_2^-$ -producing bacteria in the presence of tubificids was observed in the soil (Fukuhara et al 1980). Tubificids also increased the density of anaerobic bacteria in general and sulfate-reducing bacteria in particular in the floodwater (Kikuchi and Kurihara 1982).

#### 5.4.4. Effect on plankton and macrophytic algae

Experiments showed qualitative and quantitative effects of tubificids on algae. *Oscillatoriaceae* and *Euglena* were dominant in their presence; *Oscillatoriaceae* and *Spirogyra* were dominant in their absence. Populations of microalgae (*Chlamidomonas* sp., *Euglena* sp.), macrophytic algae (*Hydrodictyon reticulatum*, *Chara* spp.), and floating macrophyte (*Lemna pauciscostata*) were higher in plots with tubificids (Kikuchi and Kurihara 1982).

The presence of tubificids also increased zooplankton abundance (*Moina* sp., *Simocephalus* sp., and Ostracoda) (Kikuchi et al 1977, Kikuchi and Kurihara 1982). That was obviously due to the increase in phytoplankton abundance.

As a result of higher planktonic activity, N, C, and  $\text{Fe}^{++}$  contents increased in the first centimeter of soil. A concentration of  $\text{Fe}^{++}$  about twice as high in the upper soil was interpreted as the result of a faster decomposition of the OM at the soil surface in the presence of tubificids (Kikuchi and Kurihara 1982).

A major effect of tubificids is mixing and aeration of the soil, as shown by experiments where tubificid addition affected weed and zooplankton abundance, pH, Eh, and soil  $\text{O}_2$  uptake, in much the same way as artificial aeration-mixing of the soil by bubbling 500 ml of air into the soil every other day (Kikuchi et al 1977). By releasing excreta at the soil surface, destroying the oxidized layer, and stimulating OM decomposition, tubificids allow the transfer of OM,  $\text{NH}_4^+$ ,  $\text{Fe}^{++}$ , and  $\text{PO}_4^-$  and soil bacteria to the floodwater, which increases the activity and the biomass of bacteria and aquatic flora and fauna. The resulting positive feedback increases or maintains the tubificid population (Fig. 5.3).

Grant and Seegers (1985a) also showed that tubificids increased soil N uptake by rice plants and stimulated N losses from buried algae cells.

Marine and lake ecologists recognize tubificids as aerating agents, increasing oxidized layer depth and stimulating denitrification (Fry 1982, Malcolm and Stanley 1982). In ricefields, the activities of tubificids have an opposite effect, decreasing Eh, and stimulating  $\text{NH}_4^+$  formation and Fe reduction. This difference may be partly explained by the percolation effect described by Takai et al (1974). In OM-poor soils (and sediments), percolation oxidizes the upper part of the soil and, by removing water-soluble substrates, depresses microbial activities. This leads to lower production of  $\text{Fe}^{++}$  and increased Eh of the upper soil where most  $\text{O}_2$  is consumed. In OM-rich flooded soils such as rice soils, percolation, by increasing  $\text{O}_2$  availability and removing toxic substances,

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were reported to feed on rice seedling roots, but that is probably very limited in time and space (Clement et al 1977, Barrion and Litsinger 1984).

Large snails can be detrimental to the rice crop, especially the golden apple snails — an ill-defined group including several species such as *Pomacea insularis*, *P. caniculata*, *P. vigas*, *Ampullaria gigas*, and *Pila leopoldvillensis*. Golden apple snails are an important rice pest in both irrigated and rainfed environments in Surinam; Taiwan, China; Japan; and the Philippines.

In Surinam, according to Kenmore (cited in Acosta and Pullin 1991), the golden snail was not a major problem in the small-scale farming systems because the farmers controlled it effectively by hand-picking. But when large-scale, mechanized, irrigated rice production schemes were established in the 1950s, snails proliferated and became a pest. One possible reason was the decline of natural enemies with intensive, large-scale monoculture of rice in a limited environment.

In Taiwan,  $15 \times 10^4$  ha of ricefields were infested with *Pomacea canaliculata* in 1986, of which  $9 \times 10^4$  ha were treated with molluscicides. Losses were estimated at about \$31 million (Mochida 1988).

In the Philippines, the golden snail was introduced as a possible livelihood project by national and nonprofit organizations from 1980 to 1983. Then the private sector attempted to develop golden snail culture into a viable industry. At the time of introduction, the export potential of the species as escargot was well publicized, but the potential is now thought to have been largely imaginary (Acosta and Pullin 1991). Golden snail in the Philippines has led to severe adverse environmental impacts. Rice farmers first expressed alarm in northwestern Luzon in 1986; by 1988, more than  $40 \times 10^4$  ha were infested. Since then, the golden snail has become a major pest spreading all over the country.

The problems of golden snail infestation in rice farming include damage to the rice plant, yield losses, additional expenses, side effects of molluscicides, and destructive effects on the nondetrimental edible native snail *Pila luzonensis* (Basilio 1991). Golden apple snails can be very detrimental for the following reasons.

- They multiply rapidly and grow fast. They attain maturity at 3-3.5 cm and can reach 5 cm within 1 yr. An egg cluster (25-500 eggs) hatches in 8-15 d with 80% success.
- They are very resilient. They can withstand low dissolved O<sub>2</sub> in water and organic pollution. They can survive out of water because of their breathing organs. They can estivate when fields are drained, surviving up to 8 mo.
- They cause significant damage to rice seedlings. A large snail can consume a blade of a rice seedling in 3-5 min and up to 15 g *Azolla* in 12-24 h (Saxena et al 1987). The extent of damage depends on animal size, population density, and crop growth stage. Golden snails of 1.5 cm can feed on plants up to 4 wk old. Those of 6.5 cm can feed on plants up to

9 wk old. A population of 0.5 snails/m<sup>2</sup> may reduce the tiller number by 19% at 30 DT. Loss can reach 98% at 8 snails/m<sup>2</sup> (Basilio 1991).

The golden snail is a good example of the danger of introducing an exotic organism without a preliminary assessment of the possible environmental impacts.

Rice parasitic nematodes were reviewed by Prot and Rahman (1992). More than 35 genera and 130 species of parasitic nematodes have been recorded in ricefields but not all are of economic importance. With the exception of *Aphelenchoides besseyi*, the ubiquitous white tip nematode and *Ditylenchus angustus*, which parasitize the aboveground parts of deepwater rice, all are root parasites.

Hydrologic conditions, specially the length of flooding, and rice monocropping appear to be the major factors affecting the distribution of the major rice root parasites. Only a few genera and species survive in soils subjected to long flooding, although diversity is high in well-drained soils. *Hirschmanniella* spp., the rice-root nematodes, and *Meloidogyne graminicola* are ubiquitous in irrigated and deepwater ricefields where they may cause significant yield loss. It has been estimated that *Hirschmanniella* spp. cause 25% yield loss on 60% of the world's irrigated rice area. Most rice parasitic nematode pests can be controlled by chemicals or crop rotation. These control methods, however, are often uneconomical and impractical (Prot and Rahman 1992).

### 5.5.2. Effects on vector-borne diseases

Wetland rice culture and irrigation schemes in tropical and subtropical regions create ecological conditions favorable to the propagation of diseases whose vectors require an aquatic environment (Table 5.5). In particular, the presence or absence of aquatic plants is one of the ecological factors that determine the suitability of a habitat for mosquitoes and snails (Mather and Trinh 1984). Aquatic plants provide shade, shelter, resting places, and refuges from natural predators, and can also modify temperature and increase O<sub>2</sub> concentration in water.

Numerous studies have shown the association of wetland rice culture with vector-borne diseases in various parts of the tropics. But the relationships between rice culture and vector-borne diseases are complex and often highly site-specific.

Although almost all the important mosquito vectors that transmit Japanese encephalitis breed primarily in ricefields (Pant 1979, cited by Gratz 1988), those that transmit malaria or schistosomiasis do not. In the case of malaria, one of the reasons for the complexity is that several anopheline mosquito species, which are the vectors of the disease, breed in ricefields but the environmental conditions suitable for the breeding of various species differ within the overall ricefield ecosystem.

Introduction of irrigated rice creates greater mosquito breeding surfaces. At the same time, however, the ecosystem undergoes significant changes that

either favor or discourage the ecological niches required for the breeding of certain species. Such changes include duration of flooding, water depth, water turbidity, temperature, and shading pattern. These changes may shift the equilibrium of the mosquito population and species selection in the area, resulting in a decrease or increase in the transmission potential, depending on which species find the changed ecosystem favorable or unfavorable for breeding.

Gratz (1988) pointed out that it is difficult to determine how much of anopheline mosquito breeding actually takes place in ricefields. Ricefields in the plains of Assam, India, were found free from the dangerous anophelines (Najera 1988). Large ricefields in Malaya were found free of the local vector *Anopheles maculatus* (Muirhead-Thomson 1951). In Burkina Faso, malaria transmission was 2.5 times less intense in the rice-growing villages than in the neighboring villages (Carnevale and Robert 1987). Bradley (1988) concluded that in Africa, the role of ricefields in increasing malaria intensity is likely to be limited, but is important in prolonging the transmission season inasmuch as the larva of the key vector has extensive seasonal habitats outside the ricefields.

Sharma (1987) found that rice cultivation in India has a very weak (or no) relation at all with malaria transmission. In large parts of the country where rice culture dominated, malaria was found to be negligible or extremely low. Najera (1988) concluded that although ricefields still produce the highest densities of anophelines, rice growing today is not associated with the most serious malaria problem areas of the world.

The association of Asian schistosomiasis with rice culture has also been reported (Webbe 1988). It was found that the major breeding grounds of the schistosome snails are the irrigation and drainage ditches, rather than the ricefields per se. Garcia (1988) relates this observation to how recently the snail habitat has been used for rice cultivation and the intensity, continuity, and method of rice culture before the field investigation was conducted. In the Philippines, Garcia (1988) observed that in intensively cultivated rice areas, snails were found only in adjacent ditches or canals; in areas where fields were idle for part of the year and rice cultivation was less intensive, snail breeding occurred in the ricefields.

There is evidence, however, that the introduction of irrigated rice may increase vector-borne disease incidence. There has been a general mosquito problem, especially proliferation of anophelines, in areas where rice cultivation has been introduced (Carnevale and Robert 1987, Choumkov 1983, Webbe 1961). Rice cultivation tends to increase air humidity, which increases the longevity of mosquitoes.

On the other hand, turning swampy areas into ricefields reduces vector incidence. Snellen (1987) cited the success of periodic drainage practices to control malaria in Java, Indonesia, during the Dutch colonial time. As Najera (1988) pointed out, agricultural practices were recognized early as modifiers

**Table 5.5. Major vector-borne diseases that may be related to rice cultivation (Roger and Bhuiyan 1990).**

Major agent group	Disease	Agent	Major vectors	Comments
Protozoa	Malaria	<i>Plasmodium falciparum</i> <i>P. vivax</i> <i>P. malariae</i>	<i>Anopheles</i> spp.	60 <i>Anopheles</i> spp. are vectors, 35 are considered as primary vectors. Larval stages are aquatic. May breed in standing water.
Trematodes and cestodes	Schistosomiasis	<i>Schistosoma haematobium</i> <i>S. mansoni</i> <i>S. japonicum</i>	<i>Bulinus</i> spp. <i>Biomphalaria</i> spp.	Spread by aquatic and amphibious snails.
Trematodes	Other diseases caused by trematodes			Transmitted by snails through undercooked freshwater animals.
Nematodes	Guinea-worm disease			Transmitted through defective water supplies by water-flea type crustacean.
Nematodes	Filariasis	<i>Wuchereria bancrofti</i> <i>Brugia malayi</i> <i>B. timori</i>	<i>Culex quinquefasciatus</i> <i>Anopheles</i> spp. <i>Aedes</i> spp. <i>Mansonia</i> spp.	

Arboviruses	Japanese encephalitis	<i>Culex</i> <i>tritaeniorhynchus</i> <i>C. pseudovishnui</i> <i>C. vishnui</i> <i>C. annulus</i>	Viruses transmitted mainly by <i>Culex</i> spp., but also by <i>Aedes</i> spp. <i>Anopheles</i> spp., and other mosqui- toes breeding in irrigated fields.
Arboviruses	Dengue, other encephalitis and arbovirus infections		
Nonvector -borne diseases	Leptospirosis		Especially a problem of marshy land and irrigated agriculture.

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of the malaria risk of an area, and popular wisdom recognized this potential in sayings such as “malaria flees before the plow.”

Many studies have indicated that the development of irrigation systems in Africa, mostly for rice culture, has increased schistosomiasis transmission. Webbe (1988) cited examples of such a development for Egypt, Sudan, and Ghana. He also mentioned that, in Mali, high levels of schistosomiasis are associated with rice cultivation in the floodplains of the Niger River.

There is also evidence that intensified cropping may increase vector-borne disease incidence. In Egypt, the number of mosquito vectors declined in September when ricefields dried up, while the persistence of malaria in one region (Kalyubia governorate) was suspected to be due mainly to extensive rice cultivation (Rathor 1987). Luh (1987) observed variation patterns of adult mosquitoes coinciding with rice cropping seasons. Areas with a single cropping season exhibited a single peak of mosquito abundance, whereas areas with a double cropping season exhibited two peaks. Carnevale and Robert (1987) also observed a bimodal pattern of malaria transmission in Burkina Faso, coinciding with the rice crop cycle.

Rathor (1987) reports that the change from partial to perennial cropping due to improved methods of irrigation in upper Egypt resulted in a 30-fold increase in the percentage of the population infested with schistosomiasis.

Concern about vector-borne diseases is not recent, but quantitative data on the contribution of ricefields to their transmission in different environments are limited. As a result, many speculative conclusions have appeared in the literature. Given the severity and extent of these diseases, the importance of rice culture in the tropics, and the foreseeable expansion of irrigated rice, a better understanding of the magnitude of the problem in ricefields themselves and the relationships of vectors to various rice cultural practices is imperative. That is essential before any recommendations can be made on possible control measures in the ricefield vis-a-vis its surroundings and supporting irrigation network.

## Effects of crop intensification on floodwater ecology

Modern technologies in wetland rice cultivation, which use fertilizer-responsive rice varieties, fertilizers, pesticides, and optimum water and crop management practices, have tremendously increased rice yield and production, but have profoundly modified traditional rice-growing environments. There is indirect evidence that crop intensification has probably decreased species diversity in ricefields and the number of edible outputs that a farmer obtains from his field, but the other possible environmental impacts of modern technologies are far from being fully assessed.

With regard to floodwater ecology, the greatest pressure exerted on the faunal and floral communities of ricefields is agrochemical use. Both fertilizers and pesticides significantly affect population composition and dynamics.

This chapter considers successively 1) the available information on the effects of crop intensification on the biodiversity of floodwater fauna, 2) the effects of major agrochemicals (N fertilizer and pesticides) on floodwater biology, and 3) the effects of crop intensification on floodwater ecology that may affect the long-term fertility of the ricefield ecosystem.

### 6.1. Reduction of species diversity and blooming of individual species

Traditional ricefields, some of which have been cultivated for several hundred years, might be considered as climax communities. In general, a disturbance to a stabilized ecosystem reduces the number of species while provoking *blooms* of certain others; such effects have been observed in ricefields.

However, quantitative knowledge of the long-term effects of crop intensification on species diversity is extremely scarce (Table 6.1). The only reference on the species abundance in traditional ricefields is a 1975 study by Heckman (1979) in Thailand, where 590 species were recorded in one field within 1 yr. Few records of aquatic species can be compared with Heckman's record of 183 species (Table 6.1). In a 2-yr study of pesticide application on Malaysian ricefields, Lim (1980) recorded 39 taxa of aquatic invertebrates. Single sampling by Takahashi et al (1982) in four Californian ricefields recorded 10-21 taxa. In surveys of 18 sites in the Philippines (IRRI 1985) and India (Roger et al 1985a, 1987a), the highest number of taxa recorded at one

**Table 6.1. Summary of quantitative records of species/taxa in wetland ricefields (Roger et al 1991).**

1. Number of species recorded by Heckman in 1975 in a 1-yr study of a single field in northeastern Thailand (6 samplings)

Taxon	Species (no.)	Taxon	Species (no.)
Sarcodina	31	Cyanophyta	11
Ciliata	83	Algae	166
Rotifers	50	Pteridopyta	3
Platyhelminthes	7	Monocotyledonae	25
Nematoda	7	Dicotyledonae	10
Annelida	11	Pisces	18
Mollusca	12	Amphibia/Reptilia	10
Arthropoda	146		
		Total	590

2. Number of species/taxa of aquatic invertebrates, excluding protozoas, recorded by different authors

- Heckman (1979) (species), one traditional field, 1-yr study (Thailand) 183
- Lim (1980) (taxa), 2-year study of pesticide application (Malaysia) 39
- Takahashi et al (1982) (taxa) 4 fields, single samplings (California, USA) 10-21
- IRRRI 1985 and Roger et al 1985 (species) single samplings in 18 fields with pesticide applied (Philippines and India) 2-26

3. Records of arthropod species in ricefields over one crop cycle

- Kobayashi et al (1973) study in 1954-55 of several fields by net sweeping (Shikoku, Japan) 450
- Heong et al 1991: study in 1986 of five ricefields by suction (Philippines)
  - Separate samples 146, 125, 116, 92, 87
  - Combined samples 240

site was 26; the lowest, 2. Ostracods, chironomids, and molluscs dominated the invertebrate community at most sites. By hydrobiological standards, species diversity observed in both surveys was low. Similarly, records of numbers of arthropod species in Japanese ricefields obtained in 1954-55 by net sweeping (Kobayashi et al 1973) seem to indicate a higher biodiversity than in recent data collected by Heong et al (1991) in five fields in the Philippines using a suction method usually more efficient than net sweeping. All the above data were obtained using different sampling methods and time frames. The marked decrease of the values recorded since 1975 might probably be taken as a rough indication of a decrease in total number of species after crop inten-

**Table 6.2. Edible plants and animals harvested during 1975 in a Thai ricefield (Heckman 1979).**

Species	Common name
<i>Oryza sativa</i>	Main crop
<i>Ipomoea aquatica</i>	Green vegetable
<i>Pila pesmei</i>	Large edible snail
<i>Pila polita</i>	Large edible snail
<i>Macrobrachium lanchesteri</i>	Small prawn
<i>Somaniathelphusa sinensis</i>	Crab
<i>Lethocerus indicus</i>	Large edible water bug
<i>Channa stiata</i>	Snakehead
<i>Clarias batrachus</i>	Walking catfish
<i>Anabas testudineus</i>	Climbing perch
<i>Cyclocheilichthys apogon</i>	Cyprinid
<i>Puntius leiachantus</i>	Cyprinid
<i>Puntius stigmatosus</i>	Cyprinid
<i>Esomus metallicus</i>	Cyprinid
<i>Fluta alba</i>	Swamp eel
<i>Trichogaster pectoralis</i>	Edible gourami
<i>Macrogathus aculeatus</i>	Spiny eel
<i>Rana limnocharis</i>	Frog

sification. That agrees with but does not necessarily demonstrate the generally accepted concept that crop intensification has decreased biodiversity in ricefields.

Decrease of biodiversity in ricefields was attributed not only to disturbances of the ecosystem by mechanization and use of agrochemicals but to the disappearance of permanent reservoirs of organisms in the vicinity of the fields (Fernando et al 1979).

Crop intensification has reduced the number of edible species traditionally harvested from the ricefield. Heckman (1979) reported that, in northern Thailand, 1 vegetable and 16 edible animals (snail, shrimp, crab, large water bug, fish, and frog) were collected in a single ricefield within 1 yr (Table 6.2). Such a diversity of food harvested from ricefields is now uncommon. In this study, Heckman already expressed his concern about crop intensification, "Because of the great dependence of the local human population on the aquatic community for protein, a danger exists that projects designed to increase rice production may reduce the fish-producing capacity of the ricefields, thus depriving the local farmers of an important part of their diet." Heckman quoted a study by Yunus and Lim (1971) showing that chemical treatment of ricefields in Malaysia had already significantly reduced their useful fauna. The decline of fish catches in ricefields has been attributed to the combination of double cropping, which does not give fish enough time to grow, and the intensive use of pesticides (Lim 1980).

Pesticide use may also render the edible animals growing in the floodwater unfit for human consumption. That is valid for fish and molluscs, which concentrate pesticides and toxic substances such as heavy metals. A study of the effect of composted sewage sludge as a fertilizer in rice soils (Kurihara et al 1987) showed a strong bioconcentration of Cu and Zn in snails, which rendered them unfit for human consumption. The concentration of heavy metals in the flesh and the size of the shell were negatively correlated.

Agrochemical use, besides increasing rice yield and decreasing species diversity, frequently leads to explosive development of single species that might directly or indirectly have detrimental effects. Fernando et al (1979) anticipated the enhancement of specific components of the ricefield fauna because of a higher algal productivity resulting from fertilizer use, and an increase in particulate organic matter (OM) resulting from soil preparation. In a study of the aquatic invertebrate community in ricefields in Selangor, Malaysia, Lim (1980) found that species diversity decreased after pesticide application, but the overall population increased because, inter alia, of a rapid recruitment of ostracods.

In their surveys of 18 sites in the Philippines and India, Roger et al (1987a) found that 1) population dominance was inversely proportional to diversity, and 2) ostracods, chironomids, and molluscs dominated the invertebrate community at most sites, with a few species attaining exceptionally high densities ( $2.5 \times 10^3$ - $28 \times 10^3/m^2$ ) at some sites.

Examples of explosive developments of single species and their potential detrimental effects were listed by Roger and Kurihara (1988):

- Blooms of green algae and diatoms observed at the beginning of the crop after fertilizer application—may cause N losses by volatilization.
- Proliferation of ostracods observed after carbofuran application — may inhibit the development of efficient N<sub>2</sub>-fixing cyanobacteria blooms.
- Very dense populations of aquatic snails, which develop at the beginning of the crop—some are vectors of bilharziosis, others damage young rice seedlings (*Pomacea* spp).
- Large populations of mosquito larvae develop in shallow water in fertilized ricefields, but are absent in traditional ricefields where floodwater is deeper and predators are abundant (Heckman 1979)—mosquitoes are vectors of malaria, their larvae graze on N<sub>2</sub>-fixing cyanobacteria.
- Blooms of some species of aquatic weeds such as *Chara* or *Nitella*—may reduce yield.

Microalgae and aquatic invertebrate blooms usually do not last long. Only mucilaginous cyanobacteria and aquatic macrophytes may develop long-lasting, large biomasses.

When considering data on biodiversity, one must keep in mind that maintaining or increasing general biodiversity per se may not be sufficient to promote ecosystem stability and productivity in ricefields. For example, a study

of the arthropod diversity in ricefields in the Philippines has shown that high biodiversity is not sufficient to maintain low pest populations. Of the five sites where samples were collected, the IRRI experimental farm had significantly higher biodiversity, but the pest population was also significantly higher as well. Lowest biodiversity and lowest phytophage populations were recorded in the traditional rice terraces where rice has been grown with no agrochemicals for centuries (Heong et al 1991).

## 6.2. Nitrogen fertilization and floodwater ecology

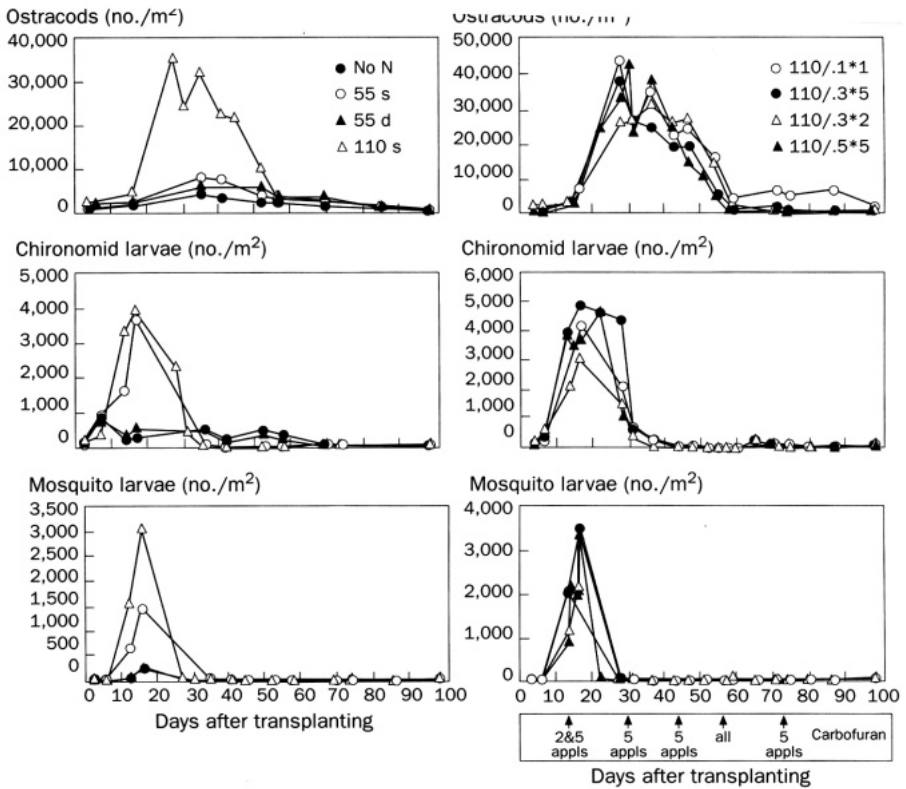
Nitrogen fertilizer application, especially broadcasting into the floodwater, increases the production of rice and the photosynthetic aquatic biomass (PAB). That in turn favors populations of primary and secondary consumers, which over the long term may increase soil fertility. Broadcasting N fertilizer, however, leads to effects contradictory to the goal of fertilization, which is to enrich the environment in N nutrient available to the plant. They include losses of applied N by  $\text{NH}_3$  volatilization (discussed in sect. 4.6.4) and direct and indirect inhibition of biological  $\text{N}_2$  fixation (BNF).

### 6.2.1. Effects on floodwater productivity

The effects of broadcasting N fertilizer into floodwater are usually obvious at the beginning of the crop cycle when there is almost no canopy, and the resulting high light availability combined with nutrient availability permits blooming of unicellular green algae and diatoms. The beginning of their growth is signaled by the formation of numerous  $\text{O}_2$  bubbles at the soil surface. Depending on the dominant algae, that may be followed either by the formation of soil-algae crusts, which get detached and float at the surface of the floodwater at the time of maximum photosynthetic activity, or by the development of a green coloration of the floodwater when motile algae are dominant. Algal growth causes marked changes in  $\text{O}_2$  content and pH of the floodwater (cf. Fig. 3.5). Fertilizer application around panicle initiation does not cause such an algal growth because 1) the larger canopy reduces light availability for photosynthetic activity in the floodwater, and 2) N uptake by the crop is more rapid.

There is evidence that chemical N fertilizer application increases rice residues remaining in soil and PAB and leads to a higher soil N fertility. A long-term fertility experiment in flooded rice soils at Konosu (Japan), from 1925 to 1979, showed higher total soil N and available N in plots where chemical fertilizer was applied than in nonfertilized plots (Kimura et al 1980). At the end of the experiment, rice was grown without added N fertilizer in all plots. Nitrogen absorbed by the crop was 48 kg/ha in previously nonfertilized plots and 54 kg/ha in previously fertilized plots. Kimura et al (1980) concluded that the larger OM supply in fertilized plots (due to a larger biomass production) may explain the larger accumulation of total and available N in

the fertilized plots than in the nonfertilized plots. That agrees with observations by Sayeki and Yamazaki (1978) who estimated the quantity of stubble and roots left after harvest of rice to be 1.4 t dry weight (dw)/ha in fertilized plots and 1.0 t dw/ha in nonfertilized plots. In addition to rice stubble and roots, weed biomass grown during the fallow period and incorporated before transplanting rice was 0.16 t dw/ha in nonfertilized plots and 1.3 t dw/ha in fertilized plots. On the other hand, during the rice crop, weed biomass was larger in nonfertilized plots than in fertilized plots, presumably due to weed suppression by the dense rice canopy in fertilized plots. However, the yearly total of weed biomass production was larger in fertilized plots (Sayeki and Yamazaki 1978).



6.1. Dynamics of zooplankton populations in plots receiving various levels of N fertilizers and pesticides. 55s = 55 kg N/ha split broadcast; 110s = 110 kg N/ha split broadcast; 55d = 55 kg N/ha deep placed; 1\*1= one application of 0.1 kg carbofuran/ha; 3\*5 = 3 applications of 0.5 kg/ha each, etc., appls = applications (Simpson et al 1994b).

### 6.2.2. Effects on floodwater fauna

The blooming of unicellular algae resulting from fertilizer broadcast into the floodwater is generally followed by the proliferation of invertebrates that graze on such algae (ostracods and chironomid and mosquito larvae) (Simpson et al 1993b) (Fig. 6.1). That might, in turn, favor the development of secondary consumers, but this aspect is not documented.

### 6.2.3. Effects on biological N<sub>2</sub> fixation

A general trend observed with cultures of N<sub>2</sub>-fixing microorganisms is the inhibition of their N<sub>2</sub>-fixing activity by chemical sources of N. In situ this inhibition is not as clearly marked. Free-living phototrophs (cyanobacteria) seem to be more susceptible to inhibition by N fertilizers than heterotrophs.

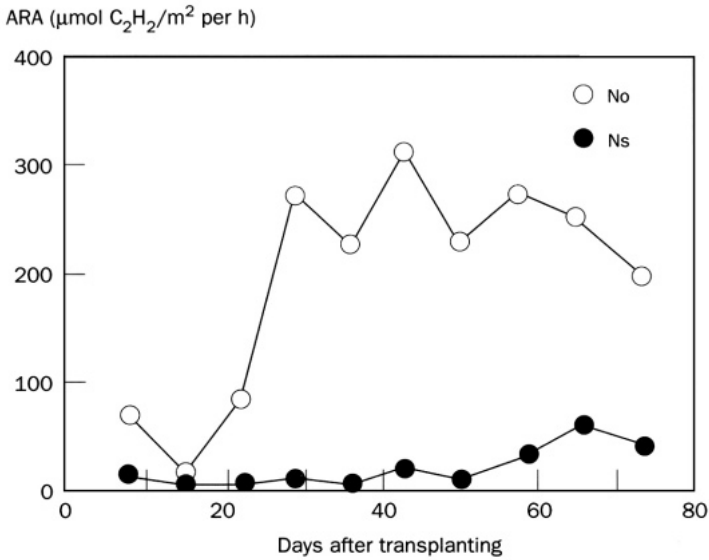
Algal populations, which develop after N fertilizer is broadcast, are dominated by eukaryotic microalgae. Surveys in long-term fertility plots showed that N fertilizer application strongly suppresses cyanobacteria populations and their bloom (Watanabe et al 1977b). Acetylene-reducing activity measurements confirm the inhibitory effect of surface application of N on photodependent BNF (see sect. 4.6.1.2). Inhibition, although never total (Table 6.3), was observed when urea was applied basally as well as when it was applied later in the crop cycle (Fig. 6.2).

After broadcast N fertilization, N in the floodwater returns to its original concentration within a few days (Fig. 3.6); therefore cyanobacteria inhibition by broadcast N fertilizer might be mostly indirect. A partial explanation is that N fertilizer favors the growth of unicellular green algae early in the crop cycle. That permits the establishment of grazers that first cause the disappearance of green algae and then might further inhibit cyanobacteria growth when the N concentration in the floodwater decreases to a level insufficient to inhibit them either directly or indirectly, through competition with green algae (Fig. 6.3). The validity of that hypothesis, however, has not been fully demonstrated.

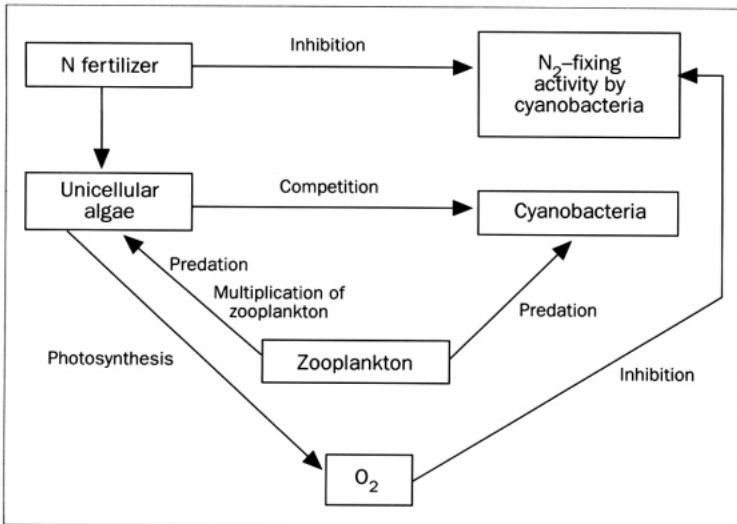
**Table 6.3. Average acetylene-reducing activity (ARA) during the crop cycle and rice yield under different urea management practices (Roger et al 1988).<sup>a</sup>**

Treatment	ARA ( $\mu\text{mol C}_2\text{H}_2/\text{m}^2$ per h)	Grain yield (t/ha)
Control (no N applied)	195 $\pm$ 14	4.08 $\pm$ 0.10
38 kg N/ha broadcast at transplanting $\pm$ 17 kg N/ha at panicle initiation	80 $\pm$ 13	4.82 $\pm$ 0.12
55 kg N/ha deep-placed at transplanting	116 $\pm$ 16	5.78 $\pm$ 0.09

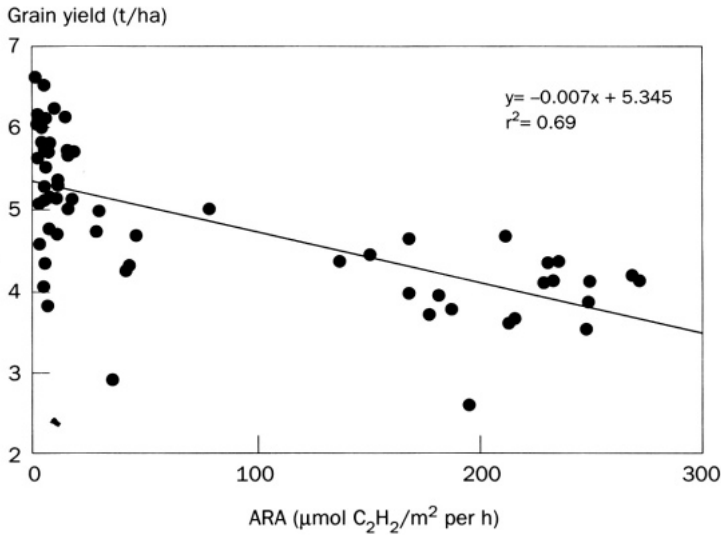
<sup>a</sup> Each value is the average of 60 data.



**6.2.** Dynamics of photodependent acetylene-reducing activity (ARA) in a field with split surface application of urea (Ns) and a control with no N fertilizer applied (No). IRRI, 1986 dry season (P. M. Reddy and P. A. Roger, IRRI 1986, unpubl.).



**6.3.** Direct and indirect inhibition of N fertilizer on N<sub>2</sub>-fixing cyanobacteria.



**6.4.** ARA and grain yield in plots where 55 kg N/ha was split broadcast (Roger et al 1988).

Photodependent BNF by free-living cyanobacteria has a potential impact of 20-30 kg N/ha on rice production where farmers do not use chemical N fertilizer. Its inhibition by chemical N fertilizers lead to the waste of a free natural input, which could be partly avoided by deep-placing or incorporating N fertilizer (see sect. 7.1.2).

Experiments at IRRI show a negative correlation between photodependent N<sub>2</sub> fixation and rice yield in plots where N was surface-applied (Fig. 6.4). It confirms the inhibition of BNF by N fertilizer and indicates that high N<sub>2</sub>-fixing activity observed after N application is an index of a poor N fertilizer efficiency, most probably because of significant losses by NH<sub>3</sub> volatilization. The low fertilizer efficiency leads to a less dense canopy and a resultant higher availability of light in floodwater, which favors cyanobacteria growth.

### 6.3. Effects of pesticides on nontarget flora and fauna

A wide range of pesticides is used in wetland ricefields to control rice pests and weeds. Nontarget organisms in water include 1) cyanobacteria and microalgae, which are involved in the maintenance of soil fertility; 2) invertebrate populations, which contribute to recycling and translocation of nutrients in floodwater and soil; and 3) invertebrates that are vectors of human and animal diseases.

### 6.3.1. Pesticide behavior in flooded soils

As in any cultivated soil, the metabolism and biological effects of pesticides depend on soil properties, climatic factors, the method of application, and synergistic/antagonistic effects among agrochemicals. Pesticide behavior in ricefields, however, is specific to wetland conditions.

In uplands, pesticides remain at the soil surface until cultivation or watering incorporates them into the soil. In wetlands, because of floodwater and puddled soil, a faster dilution can be expected, with variations depending upon solubility and surfactants used. The various combinations of water management and pesticide application might affect pesticide toxicity with regard to dilution and movement in soil, but no information is available on this aspect. Pesticide application methods (spraying, floodwater application, incorporation into soil, dipping of seedlings) alone can induce significant differences in pesticide behavior. Pentachlorophenol, incorporated into soil with  $\text{CaCO}_3$  stimulated  $\text{N}_2$ -fixing cyanobacteria. But if it was surface-applied, even at low levels, it suppressed cyanobacteria growth and exhibited a long residual effect (Ishizawa and Matsuguchi 1966). The pesticide HCH suppressed rhizospheric BNF when it was incorporated, but stimulated it throughout crop growth when applied to the floodwater (Rao et al 1983).

Pesticide degradation in flooded soils results from microbiological activities, chemical transformations, and volatilization, including gaseous exchanges between the soil and the atmosphere through the rice plant.

Microbial degradation in tropical ricefields is favored by 1) temperatures and pH, which usually stabilize in a range favoring microbial activity, and 2) reducing conditions caused by submersion. Decomposition is further accelerated by OM incorporation. As a result, pesticide degradation is often faster in flooded than in nonflooded soils (Sethunathan and Siddaramappa 1978). In uplands, bacteria and fungi are considered to be mainly responsible for pesticide transformations. In wetlands, fungi are probably less important, whereas the role of algae may be significant as shown for parathion (Sato and Kubo 1964). The relative importance of nonbiological degradation varies with pesticides and environmental conditions. For various insecticides, it ranged from 30 to 90% of the degradation in soil estimated in the presence of microflora (Agnihotri 1978). Degradation of carbofuran in water was mainly by nonbiological process(es) and was related to the initial pH, but in soil it was associated with microbial activities (Siddaramappa and Seiber 1979).

Besides microbiological and chemical degradation/transformation, pesticides can disappear from the ricefield through volatilization (Soderquist et al 1977). Pesticide losses by volatilization can be retarded in flooded environments, as shown for trifluralin (Parr and Smith 1973).

### 6.3.2. Methodological aspects

Roger et al (1994) compiled and analyzed a comprehensive, bibliographic data base of 547 references on the impacts of pesticides on microorganisms grow-

**Table 6.4. Characteristics of a bibliographic data base on microbiological impacts of pesticides in ricefields (Roger et al 1994).**

	Number of references	
<i>Main topics</i>		
Methodological aspects including bioassays		13
Decomposition and persistence of pesticides in rice soils		140
Effects on heterotrophic microbial populations and activities		91
Effects on algae and cyanobacteria		272
Algicides and algal weeds	38	
Quantitative effects on growth and activities	149	
Qualitative effects	29	
Bioconcentration in algae	13	
Effects on algal grazers	11	
Effects on symbiotic BGA ( <i>Azolla</i> )	5	
Adaptation and resistance of algae to pesticides	27	
Miscellaneous		26
Reviews including references to wetland soils		18
Total		547
Algological studies including cyanobacteria		
Type of experimental design	(no. of reports)	Bacteriological studies (no. of reports)
<i>Methods used</i>		
Cultures of microorganisms	130	2
Cultures of microorganisms with soil	6	0
Soil in test tubes or beakers	0	24
Pot experiments	3	21
Field experiments	10	14
Method not available	0	10
Total	149	71

ing in, isolated from, or known to be present in ricefields (Table 6.4.). About half of the collected papers presented quantitative estimates of the effects of pesticides on microbial populations or their activities. The analysis showed that available information is questionable for quantitative analysis and interpretation because of 1) bias in the nature of organisms and pesticides tested, 2) the methods adopted, and 3) the pesticide concentrations used.

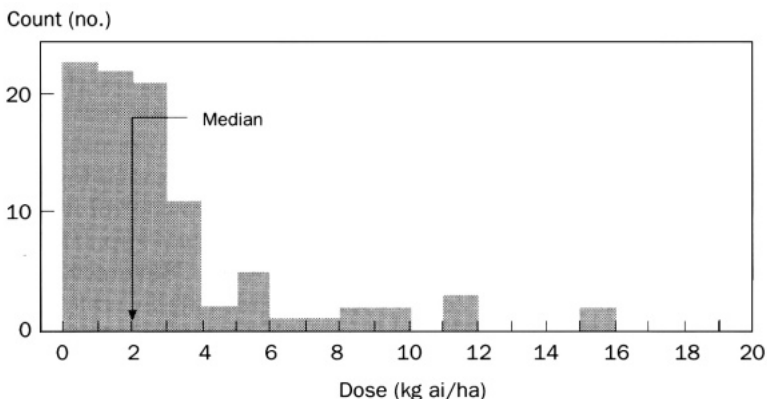
6.3.2.1. *Representativeness of the materials.* Organisms and pesticides that have currently been studied do not constitute a representative sample of the many possible combinations of microorganisms and pesticides that occur in ricefields. The literature on microalgae is much more abundant than that on other microorganisms and deals mostly with herbicides (62% of the records) and cyanobacteria. The literature on other microorganisms deals mostly with insecticides (80% of the records).

6.3.2.2. *Methods.* Most studies are small-scale laboratory experiments consisting of toxicity tests with algal cultures, or test tube or flask experiments

with a few grams of soil (Table 6.4.). Less than 8% of the quantitative studies were conducted in situ. Experiments with cultures of microorganisms give an index of a strain's sensitivity to pesticides. Results obtained in vitro, however, can hardly be extrapolated to field conditions for the following reasons.

- Toxicity is likely to be higher in culture than in situ where pesticide degradation is enhanced by soil microflora, nonbiological decomposition, leaching, volatilization, and soil absorption. For example, 5 ppm propanil prevented the growth of cyanobacteria in culture, but not in the presence of unsterilized or sterilized soil and in situ (Ibrahim 1972, Wright et al 1977).
- Toxicity depends on the initial population, its nutrient status, and the method of pesticide application. These conditions are likely to differ markedly in vitro and in situ.
- In vitro experiments often test pure ingredients, while in situ toxicity depends on the formulation. Some additives used as surfactants in commercial formulations are detrimental to algae or enhance the effect of pesticides (Arvik et al 1971).
- In situ toxicity also depends on degradation products, some of which can be as inhibitory or even more so than the parent compound, as shown for atrazine (Stratton 1984, Wright et al 1977). Metabolic products of aldrin, dieldrin, and endrin inhibit algal growth (Batterton et al 1971). 3-4 dichloroaniline, the primary product of propanil degradation, is less inhibitory than propanil, but at the concentration of propanil used in the field (12 ppm), the degradation product can still inhibit the growth of some cyanobacteria (Wright et al 1977).

6.3.2.3. *Pesticide level.* Many in vitro studies and some field studies were conducted with pesticide concentrations far higher than those resulting from the recommended level for field application. Such studies are of little value for drawing conclusions, except when no significant effect was recorded. Recommended level for field application of the 150 chemicals that have been tested for their microbiological impacts in ricefields range from a few hundred grams to a few kilograms active ingredient (ai)/ha, with a median of about 2 kg (Fig. 6.5). When interpreting experimental results, one should keep in mind the range of pesticide concentrations that can be expected in farmers' fields after application. The upper limit can be calculated by assuming that pesticide is applied on a nonflooded soil and remains in the first 2 cm. In this case, 1 kg ai/ha corresponds to 10 ppm on a dry soil basis (bulk density 0.5). A lower limit can be calculated by assuming an even distribution in 10 cm of water and 10 cm of puddled soil of a water-soluble pesticide. In this case, 1 kg ai/ha corresponds to 0.4 ppm. The field situation is probably closer to the lower value. Concentrations of 10-500 ppm often tested in vitro appear to be used more to estimate a relative lethal concentration ( $LC_{50}$ ) than to reflect the actual field situation.



**6.5.** Histogram of the average recommended doses of 94 pesticides tested for their microbiological effects in ricefields (Roger et al 1992).

### 6.3.3. Effects on algae

This section presents primarily the analysis of the algological data from the data base used by Roger et al (1994) for their review of microbiological impacts of pesticides in ricefields.

6.3.3.1. *General trends.* Available data are mostly percentages of inhibition of various pesticides estimated by various measurements on cultures (dw, fresh weight, total N, chlorophyll content, etc.). The data base tabulates 1,045 records of pesticide effects on algae and cyanobacteria. It should be noted that 638 tests were performed at concentrations higher than the recommended level for field application, probably because most studies were conducted in vitro (96%) and were aimed at establishing LC<sub>50</sub> for the strains rather than testing the possible effects in situ.

Table 6.5 analyzes the 407 records of pesticide effects obtained at concentrations corresponding to the manufacturer's recommendation. An absence of pesticide effect was reported in 39% of the records and in 62% of the records obtained in situ or in the presence of soil. This confirms that pesticide effects are more marked in vitro than in situ.

In field studies, two major effects of pesticides on ricefield algae have been reported.

- A selective toxicity, which affects the composition of the algal population. Cyanobacteria resistance to pesticides varies widely with strain. Many can tolerate high levels of pesticides, higher than those generally recommended for field application. Cyanobacteria are often more resistant to pesticides than eukaryotic algae, which may lead to a selective effect of pesticide in favor of cyanobacteria.
- A growth-promoting effect of insecticides due to the decrease of invertebrate populations that graze on algae.

Several references confirm that ricefield algae can significantly contribute to the bioconcentration of pesticides just as many microalgae in freshwater environments do. That is important when considering the ricefield ecosystem as a possible environment for aquaculture (rice-fish, rice-shrimp).

6.3.3.2. *Effects of algicides and fungicides.* Many fungicides for use in ricefields were tested primarily as algicides and are therefore considered together with algicides. Algicides are usually applied to ricefields to control macrophytic (*Chara* spp., *Nitella* spp.) or mat-forming algae (*Spirogyra* spp., *Hydrodictyon* spp.). Microalgae are usually not considered as weeds. Several reports indicate a selective inhibitory effect of algicides on green algae, which promotes cyanobacteria growth. That was observed with Symetryne (Yamagishi and Hashizume 1974) and Algaedyn (Almazan and Robles 1956).

6.3.3.3. *Effects of insecticides.* Insecticides had a low impact on tested algae (mostly cyanobacteria), as shown by the high percentages of records indicating no inhibition, either when considering all experiments (67%) or considering only in situ experiments (90%) (Table 6.5).

Several reports indicated a selective inhibitory effect of insecticides on green algae, which promoted cyanobacteria growth. That was observed with BHC (Ishizawa and Matsuguchi 1966, Ragu and McRae 1967) and PCP (Watanabe 1977). Simultaneously, insecticides inhibited invertebrates that feed on algae (grazers), further promoting cyanobacteria and photodependent BNF. That was observed with parathion applied to the floodwater (Hirano et al 1955), phorate (Srinivasan and Emayavaramban 1977), and carbofuran (Tirol et al 1981).

**Table 6.5. Summarization of the data on the effect of 109 pesticides on ricefield microalgae at concentrations corresponding to the recommended level for field application (Roger et al 1993).**

Nature of data	Data (no.)	Percentage of data corresponding to each of the five levels of inhibition (i)				
		i = 0	0 < i < 50%	i = 50%	50% < i < 100%	i = 100%
All data	407	39	19	26	2	14
All data in situ or with soil	39	61	8	3	3	25
Algicides (3 tested)	33	3	0	67	0	30
Fungicides (22 tested) <sup>a</sup>	30	40	10	7	0	43
Herbicides (57 tested)	252	33	25	28	2	12
Herbicides in situ or with soil	24	58	9	4	4	25
Insecticides (28 tested)	97	67	12	14	3	4
Insecticides in situ or with soil	10	90	10	0	0	0

<sup>a</sup> Several fungicides act also as algicides.

Insecticide application did not invariably increase photodependent BNF. Some inhibitory effect was reported for PCP in situ (Ishizawa and Matsuguchi 1966). Also, over the long term, insecticide use might become detrimental to cyanobacteria by decreasing the diversity of aquatic invertebrates and causing algal grazers resistant to conventional pesticides to proliferate (see sect. 6.3.4).

6.3.3.4. *Effects of herbicides.* Among pesticides not aiming at algal control, herbicides seem to be the most detrimental to algae, causing partial or total inhibition in 67% of the in vitro tests and in 42% of the tests performed in situ or in the presence of soil (Table 6.5). Herbicides can inhibit cyanobacteria and photodependent BNF, as shown with PCP, used both as an insecticide and herbicide (Ishizawa and Matsuguchi 1966), and several formulations used in ricefields (Srinivasan and Ponnuswami 1978). Some herbicides seem to affect specifically the N<sub>2</sub>-fixing ability of cyanobacteria as indicated by an inhibition observed in N-free medium, but not in the presence of inorganic N. That was observed with dichlone (fungicide/algicide) (Kashyap and Gupta 1981) and butachlor (Kashyap and Pandey 1982).

6.3.3.5. *Effects of pesticides on free-living and symbiotic bacteria used as biofertilizers.* In numerous experiments on inoculation of ricefields with algae (see sect. 7.4), very few trials have tested the interaction between herbicides and algae. Kerni et al (1984) concluded that butachlor applied to inoculated plots at the rate of 5-30 kg/ha had no effect. In a pot experiment, El-Sawy (1984) showed that when algal inoculation was effective, herbicide application most often had either no effect or a positive effect over the inoculated control (14 of 16 cases). Negative effects (2 of 16 cases) were observed with propanil.

The effects of both herbicides and pesticides have been tested on *Azolla*. Since one of the benefits of *Azolla* use is reduced weed incidence by competition (see sect. 7.31, it is doubtful that herbicides will be used together with *Azolla*. The information on herbicide impacts on *Azolla* is useful for assessing the effect of pesticides on small macrophytes.

Field experiments showed detrimental effects of herbicides on *Azolla* growth (Janiya and Moody 1981a, Lales et al 1986, Singh et al 1988). Holst et al (1982) tested 15 pesticides on *Azolla mexicana*. Bipyrilidium and phenolic herbicides were the most detrimental, reducing BNF up to 75% at 0.1 ppm. Chloramben and benomyl at 10 ppm reduced BNF 84-99% without affecting growth. Growth and BNF were reduced by other benzoic, triazine, dinitroaniline, and urea herbicides tested at 0.1-10 ppm. Naptalam was the only pesticide tested that had no effect on growth or BNF by *Azolla* at 10 ppm. Preemergence herbicides seem to be less inhibitory; 2,4-D was reported not to affect *Azolla* even when applied together with the inoculum. The inhibitory effect of some herbicides may be reduced or avoided entirely by the appropriate timing of application (Lales et al 1986).

Threshold values for toxicity are much higher for insecticides (carbofuran: 0.5 ppm; lindane: 0.1 ppm) than for herbicides (2,4-D : 0.05 ppm; butachlor : 0.001 ppm) (Singh et al 1984). Insecticides favor *Azolla* growth by decreasing insect pest incidence, but they may also be phytotoxic. A study of nine insecticides showed higher toxicity on *Azolla pinnata* than on *A. microphylla* and higher toxicity of sprays than granular formulations (Mochida 1987).

#### **6.3.4. Effects on fauna**

In vitro effects of pesticides applied in ricefields on soil and water fauna include acute toxicity, alteration of filtration and assimilation rates, and inhibition of growth and egg production. But herbicides, fungicides, and molluscicides appear to possess limited toxicity to invertebrates at the concentrations in which they are applied in the field (Georghiou 1987). Insecticides are the most active pesticides on floodwater invertebrates.

6.3.4.1. *Methodological aspects.* A few field experiments studied the impact of pesticides on specific components of the zooplankton, but records of the dynamics of the major components of the floodwater biota during a crop cycle and under a range of agricultural practices are scarce. Data for both temperate (Kurasawa 1956, Ishibashi and Itoh 1981) and tropical ricefields (Simpson et al 1993a,b; 1994a,b,c) are available.

Few experiments were conducted in farmers' fields or in fields managed according to farmers' usual practices. In many published works where effects on aquatic invertebrates have been reported, however, experimental pesticide application rates have been higher than the recommended one. Such information is useful in the context of pesticide misuse and accidental spillages, but where pesticides are used judiciously, the findings are of limited value. Experimental designs testing effects of pesticides in the absence of fertilizer are also of limited value because that situation is rare in farmers' fields. There is also evidence that when both N fertilizer and pesticides are applied at the recommended dose, zooplankton are affected more by the fertilizer than by the pesticide (Simpson et al 1994b). Finally, in most earlier studies, no statistical analysis of the data was performed. Inasmuch as zooplankton exhibit contagious distributions, marked differences are not necessarily statistically significant.

6.3.4.2. *Effects on zooplanktons.* The major reported effect of insecticides applied in ricefields on zooplankton is decreasing species diversity and the blooming of individual species, especially ostracods, mosquito larvae, and molluscs (Roger and Kurihara 1988) as populations of predators such as Odonate larvae decrease. There are also reports indicating that pesticides have a limited effect on zooplankton.

In Japan, Takaku et al (1979) observed that the application of fenitrothion by helicopter greatly reduced the abundance of *Moina* sp. in floodwater. Ali (1990) reported that rotifer, cladoceran, and copepod populations in Malaysian ricefields were adversely affected when carbofuran was applied at

5.6 kg ai/ha. However, that rate is higher than any used in a survey in the Philippines (Simpson et al 1994b).

In Indonesia, Lim (1980) recorded zooplankton dynamics in 1976 and 1977 in fields where no pesticide was applied, and again in 1979 in the same fields when pesticide was applied. He found that nematodes, hemipterans, and dipterans dominated when fields were not treated whereas ostracods, dipterans, and conchostracans dominated in fields when pesticides were applied at the recommended dose. Total zooplankton decreased from 1,500/liter to 400/liter after carbofuran application and recovered within 2 wk. Zooplankton were more abundant in pesticide-treated fields partly because of a rapid recruitment of ostracods. Ostracods and molluscs were also reported to dominate in insecticide-treated fields of the Philippines (IRRI 1985). A field study by Takamura and Yasuno (1986) reports the development of large populations of chironomids and ostracods in herbicide- and insecticide-treated fields. Simultaneously, the number of natural predators of chironomids and ostracods decreased. Benthic algae decreased in herbicide-treated plots and did not increase in insecticide-treated plots, probably because of grazing by ostracods. The pesticide concentration used in this study was not indicated.

Lim and Wong (1986) attributed the dominance of ostracods in pesticide-treated fields to their resistance to pesticides and the large number of eggs produced parthenologically. The  $LC_{50}$  to *Heterocypris luzonensis* of carbofuran was 2.4  $\mu\text{g/ml}$  and, of lindane, 56.0  $\mu\text{g/ml}$  (Grant et al 1983a). Such resistance to conventional pesticides allows large densities of ostracods to develop ( $0.5 \times 10^4$ - $1.5 \times 10^4/\text{m}^2$ ), particularly as the natural ostracod predators succumb first. Such populations may cause algal blooms to disappear in a few days. Ostracod activity is easy to notice as the floodwater becomes muddy because of their scraping action at the soil surface.

Whereas insecticides appear to favor ostracods, Ishibashi and Itoh (1981) reported that the application of the herbicide benthocarb almost completely inhibited ostracods over the entire crop cycle.

There are reports indicating a moderate effect of pesticides on zooplankton. Lim et al (1984) observed a shift in the peaks of the cladoceran population after the application of an organophosphate pesticide, but maximum populations recorded were not affected. Lim and Wong (1986) found densities of ostracods (*Stenocypris major*) averaging 10,000 individuals/ $\text{m}^2$  in the control and  $2.1 \times 10^4/\text{m}^2$  in carbofuran-treated plots. Corresponding average productivities were 21 g dw/ $\text{m}^2$  per day in the control and 34 g dw/ $\text{m}^2$  per day in the carbofuran-treated plot. With regard to the contagious distribution of ostracods in ricefields and the fact that only duplicate measurements were performed, the statistical significance of the difference is questionable. In a field study of the effects of carbofuran at high levels of N fertilizer, Simpson et al (1994b) concluded that aquatic invertebrates were not significantly affected (Fig. 6.1).

6.3.4.3. *Effects on aquatic oligochaetes.* Available data indicate that aquatic oligochaetes are sensitive to pesticides, but they are so few that one cannot draw definite conclusions.

The appearance of significant densities of tubificids in an experimental ricefield studied for 9 yr was attributed to 1) a change in the nature of pesticides used (PCP was replaced by NIP, CNP, or benthocarb), and 2) the pollution of the irrigation water by domestic sewage. Simultaneously, N content of the soil increased by about 17% within 1 yr and grain yield by 0.9 t/ha (Kurihara and Kikuchi 1988). The study of some of the biological effects of fish stocking and pesticide use at recommended doses (insecticide carbofuran, herbicide butachlor, and molluscicide triphenyltin hydroxide) in experimental plots in the Philippines (Roger et al 1994) showed that pesticide application caused a statistically significant increase of the photosynthetic activity in floodwater and reduced average populations of aquatic oligochaetes over the cropping season from 1,760/m<sup>2</sup> to less than 200/m<sup>2</sup>. There was no significant effect on surface soil N, microbial biomass, available N, populations of N<sub>2</sub>-fixing cyanobacteria, and fish and rice yields. In a 2-yr study of the combined impacts of pesticide and N fertilizer, Simpson et al (1993a) observed a significant decrease of aquatic oligochaetes during the first crop cycle in carbofuran-treated plots, but no significant difference was observed during the second year when the same treatment was applied.

Ishibashi and Itoh (1981) found no significant effect of the herbicide benthocarb on average populations of saprophytic and parasitic nematodes enumerated on 14 occasions during a crop cycle.

6.3.4.4. *Effects on gastropods.* Snails are usually not affected by conventional rice pesticides, but their populations may increase because of reduced competition for energy sources. In India, Roger et al (1985a) observed that mosquito larvae and molluscs (*Limnea* and *Vivipara*) were either dominant or abundant in BHC-treated plots. After harvest, Ishibashi and Itoh (1981) observed larger snail populations in fields previously treated with benthocarb than in the untreated control. Simpson et al (1994c) found limited evidence suggesting that snails were favored by carbofuran or butachlor application.

6.3.4.5. *Effect on vectors of human diseases.* Most agricultural insecticides are nonspecific; they are toxic to agricultural pests as well as to some vectors of human diseases. Insecticides have three major effects on vectors: 1) they temporarily decrease their incidence, 2) they cause resurgence of resistant strains, and 3) they adversely affect natural predators and competitors of vectors.

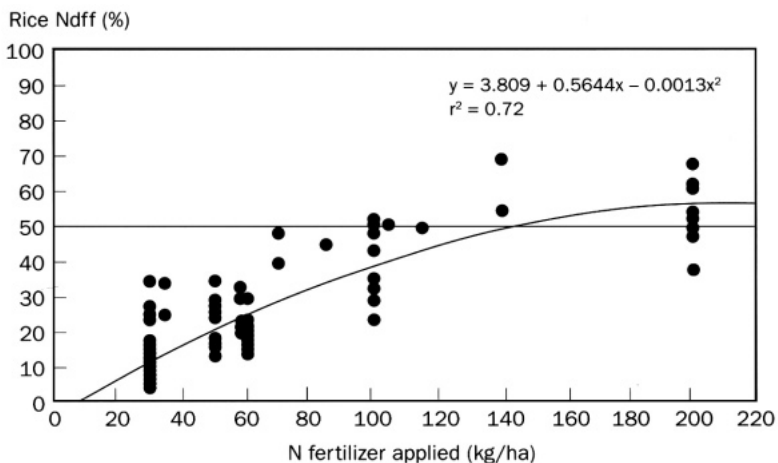
Numerous data in the literature confirm the potential of agricultural insecticides to suppress mosquitoes, larvae as well as adults, and their predators through direct toxicity (Mulla and Lian 1981). Among the probable explanations for the marked reduction of malaria and Japanese encephalitis in Japan since 1945, Self (1987) listed the significant reduction of vector populations through the extensive use of insecticides for agricultural pur-

poses. Similarly, Mogi (1987) attributes the reduction of mosquito vectors in Japanese ricefields in the late 1960s to the intensive application of organophosphate and carbamate insecticides. Self (1987) indicates that in the Republic of Korea, however, pesticide application for agricultural purpose reduced the density of Japanese encephalitis vector *Culex tritaeniorhynchus* in rice-growing areas, but had no effect on the main malaria vector *Anopheles sinensis*. The decrease of *C. tritaeniorhynchus* after 1970 in Japan is perhaps due, in part, to the increase of its natural enemies by the switch from chlorinated hydrocarbons to carbamates that do not adversely affect vector predators (Mogi 1987, Wada 1974).

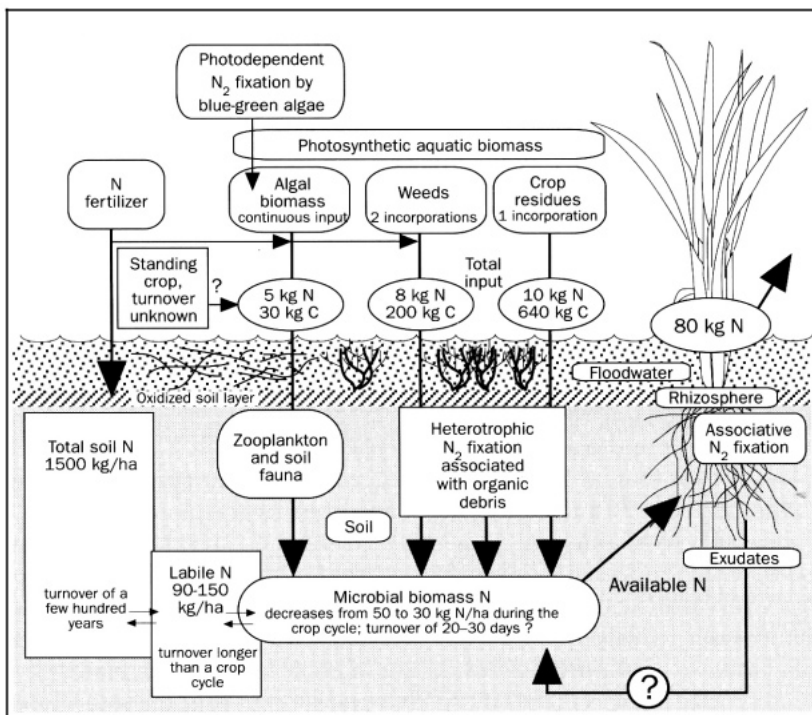
In the rice-growing areas in the USA, the elimination of malaria by suppressing the vector with DDT in the post-World War II era led the way for extensive insecticide use against both mosquitoes and agricultural pests. Chlorinated hydrocarbon, organophosphate, carbamate, and, more recently, synthetic pyrethroid insecticides have been used extensively enough in the USA to have produced resistance in some riceland mosquitoes. Experience has shown that mosquito populations are particularly adept at evolving resistant strains. A resistant strain arises because some individuals within the population already possess a genetic makeup that confers resistance on them even before their exposure to any insecticide (Mather and Trinh 1984). Observations with upland crops show a strong correlation between the intensity of pesticide use on crops and the degree of extended vector resistance. In 1987, 50 malaria vectors resistant to one or more pesticides were recorded in the world (Bown 1987).

#### 6.4. Possible effects of crop intensification on long-term soil fertility

Traditional wetland rice cultivation sustained moderate yields for thousands of years without deterioration of the environment (Bray 1986). During the last decades, yields were increased by crop intensification, the introduction of high-yielding, modern varieties, and the adoption of new technologies including pesticide and chemical fertilizer use. Research on rice nutrition, however, has shown that, at the levels of inorganic fertilizer currently applied to ricefields, between 50 and 80% of the N absorbed by the plant originates from soil (Fig. 6.6). Available soil N is released by the turnover of a microbial biomass, which represents only a few percent of total soil N (Watanabe et al 1988a). Crop residues, rhizosphere exudates, and algae and aquatic plants in the floodwater contribute nutrients that allow the replenishment of the microbial biomass. Nutrients accumulating in algae and aquatic plants (including biologically fixed N<sub>2</sub>), and in the detritus layer at the soil-water interface are recycled by the zooplankton and reincorporated into the soil by oligochaetes (Fig. 6.7).



6.6. Estimation of rice N derived from fertilizer (Ndff) at various levels of application of inorganic fertilizer (drawn from data of Broadbent et al 1987; R. J Buresh, IRRI 1990, unpubl.; IAEA 1970; and John et al 1989).



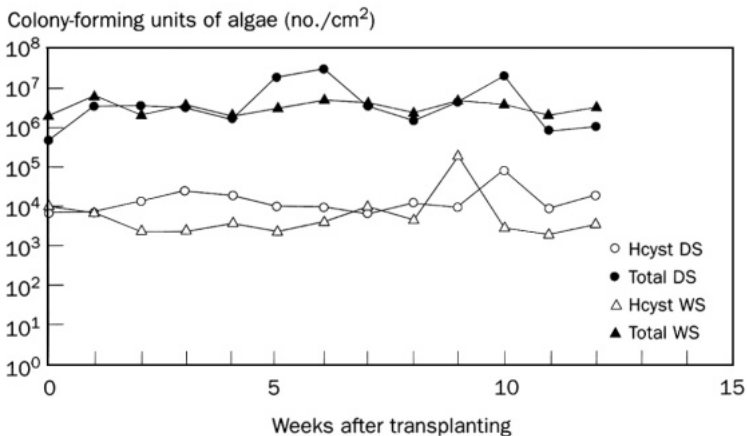
6.7. Conceptual representation of the origin of N absorbed by rice and the pathways involved in the replenishment of the microbial biomass.

The 60% increase in rice production needed for the next 30 yr will have to be obtained without a significant increase in the currently cultivated area. Therefore, it is important to understand and to predict how factors associated with crop intensification may affect the sustainability of the ricefield agroecosystem through its effects on 1) PAB, and 2) the soil and water fauna. Verifiable data on this topic are extremely fragmentary, therefore the discussion that follows is largely hypothetical.

#### 6.4.1. Effects related to the photosynthetic aquatic biomass

The PAB, which develops in ricefield floodwater, acts to some extent as a trap for C and N evolved from the soil. It helps to reduce nutrient losses when algae and weeds are reincorporated into the soil, either by weeding or by the activity of the soil and water fauna. Under intensive rice cultivation, the combination of pesticide use, intense weeding, and a dense rice canopy may restrain the growth of PAB, which in turn may affect soil fertility on a long-term basis. Three kinds of observations at IRRI possibly indicate a decrease in soil fertility when photosynthetic growth in floodwater is restrained.

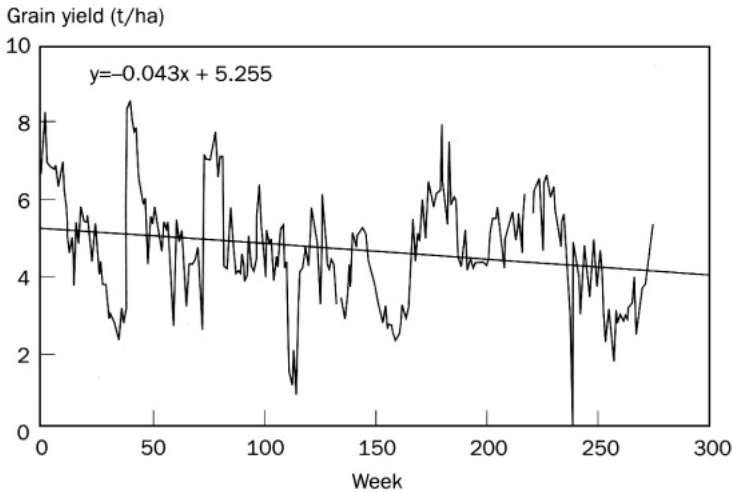
A high-input, experimental, continuous, year-round rice production system known as *the vice garden* was established at IRRI in 1978 and lasted until 1983. The field was divided into 13 small plots, equal to the rice crop duration in weeks (Domingo 1985). Each week, one plot was transplanted and another was harvested. Intensive weeding was performed and high levels of pesticides were used. As a result, PAB was very low in the plots. There was almost no algal growth in either the dry season or wet season as indicated by algal densities, which did not significantly differ from the values measured just after plowing (Fig. 6.8). Yield records from 1978 to 1983 showed productivity



6.8. Abundance of N<sub>2</sub>-fixing cyanobacteria (Hcyst) and total algae (Total) in the plots of the rice garden at IRRI as a function of the age of the crop during the 1985 wet season (WS) and dry season (DS) (P. A. Roger, S. Santiago-Ardales, and R. Remulla-Jimenez, IRRI 1985, unpubl.).

decreasing over time (Fig. 6.9). The absence of data on soil chemistry, microbial biomass, and pest incidence in the experiment precludes drawing definite conclusions on how the absence of PAB may or may not have contributed to the decrease in productivity.

The contribution of PAB to soil microbial biomass in the upper soil layer was studied in microplots filled with 4 cm unplanted soil, by comparing a dark control to soil exposed to light in which weeds and algae were reincor-



6.9. Weekly grain yield in the rice garden at IRRI from 1978 to 1983 (drawn from data of Domingo 1985).

**Table 6.6. Comparison of total N content and microbial biomass estimated by fumigation-extraction in unplanted, continuously flooded, Maahas soil exposed to light or kept in the dark for 1 yr (IRRI 1990).**

	Total N <sup>a</sup> (% of soil dw <sup>b</sup> )	Microbial biomass <sup>a</sup>	
		(ppm of soil dw)	(% of total soil N)
<i>Soil exposed to light</i>			
Initial total N content	0.168 c	...	...
After 1 yr			
1st cm	0.249 a	97.6 a	3.9 a
Deeper soil	0.168 c	51.3 b	3.0 b
<i>Soil kept in the dark</i>			
Initial total N content	0.180 b	...	...
After 1 yr			
1st cm	0.161 c	30.4 c	1.9 c
Deeper soil	0.151 c	27.7 c	1.8 c

<sup>a</sup>In a line, means followed by a common letter are not significantly different at the 5% level by DMRT. <sup>b</sup>dw = dry weight.

porated at 2- to 3-wk intervals (IRRI 1990). After 1 yr, total N content in the soil exposed to light had increased by 47% in the first centimeter and remained constant in the lower layer (Table 6.6). This increase was obviously attributable to BNF. In the soil kept in the dark, N content decreased by about 11 % in the first centimeter and 15% in the lower layer. Mineralization of microbial biomass after chloroform fumigation, followed by 4 wk incubation (fumigation-incubation method), released 98 ppm dry soil  $\text{NH}_4^+$ -N in the first centimeter of the soil exposed to light and 51 ppm in the lower layer. About 30 ppm dry weight  $\text{NH}_4^+$ -N was released in both layers of the soil kept in the dark. That showed that PAB was directly (by itself) and indirectly (through its nutrient inputs) accountable for about 40% of the soil microbial biomass in the 4-cm layer of soil.

The effects on soil microbial biomass of 1) weed incorporation, 2) weed removal, and 3) suppression of the photosynthetic activity in the floodwater were studied in a field experiment (IRRI 1990). During the first crop, flush N values (fumigation-incubation method) in the upper soil layer of the dark control were consistently significantly lower than in the soils exposed to light. Values were also consistently lower in plots where weeds were removed than in plots where they were incorporated, but the difference was not statistically significant. At the end of the second crop, a significant effect of weed removal and elimination of light was observed. Flush N was about 22% lower in the dark control than in the treatment where weeds were incorporated (Table 6.7).

These results need to be confirmed over a longer term, but they already show that PAB is a significant component of and a source of nutrients for the soil microbial biomass. They suggest that cultural practices that deprive primary producers of floodwater might affect long-term soil fertility.

#### **6.4.2. Effect on soil and water fauna**

Both N fertilizer and pesticides seem to increase zooplankton activity somewhat. On the other hand, pesticides might decrease populations of aquatic oligochaetes (see sect. 6.3.4.3). If that is true, the translocation of nutrients from the detritus layer at the soil-water interface to a deeper layer of the soil might decrease, thus reducing soil fertility. Currently no data are available to substantiate this hypothesis.

#### **6.4.3. Long-term effect of pesticides**

The knowledge on effects of pesticides in wetland soils is also too fragmentary to draw conclusions other than general trends (see sect. 6.3.2). It is important to emphasize that pesticides can significantly affect the soil-floodwater ecosystem without being detrimental. For example, a shift in algal community structure may not affect soil fertility, providing that aquatic primary production is unchanged. Therefore, one should be cautious when assessing the nature of the effects, which should be considered in the context of ecosystem equilibrium and not in isolation.

**Table 6.7. Effect of photosynthetic aquatic biomass management on soil microbial biomass in the first 7 cm of soil, estimated by chloroform treatment as ppm NH<sub>4</sub><sup>+</sup>-N (adapted from IRRI 1990).<sup>a</sup>**

Measurement	Soil depth (cm)	Microbial biomass (kg N/ha)		
		Weeds incorporated	Weeds removed	Soil and water kept in the dark
<i>Dry season</i>				
27 DT <sup>b</sup> (before weeding)	0-2 cm	47.7a	47.7a	37.5b
	2-7 cm	41.8a	41.8a	28.1b
48 DT	0-2 cm	34.3a	34.9a	28.6a
	2-7 cm	31.3a	28.9a	29.6a
76 DT	0-2 cm	36.2a	34.5a	23.6b
	2-7 cm	28.2a	27.8a	26.9a
91 DT	0-2 cm	36.4a	39.9a	25.3b
	2-7 cm	26.5a	26.2a	25.9a
<i>Wet season</i>				
2 DT	0-7 cm	37.9a	37.1a	32.9b
92 DT	0-2 cm	40.4a	33.9b	25.4c
	2-7 cm	32.7a	29.7a	28.1b
Average	0-7 cm	35.2a	34.2a	28.2b

<sup>a</sup> Microbial biomass was estimated by chloroform treatment in ppm NH<sub>4</sub><sup>+</sup>-N of fresh soil and extrapolated in kg N/ha. Each value is the average of two measurements on a composite sample of 20 cores collected in 10 replicated experimental plots. In a row, means followed by a common letter are not significantly different at the 5% level by DMRT. <sup>b</sup> DT = days after transplanting.

It would be as unwise to underestimate as to overestimate the significance of pesticide effects in wetland soil. Underestimation could cause ecological damage; overestimation could restrict the judicious use of pesticides when appropriate.

In spite of the recognized importance of the floodwater biota for soil fertility (Watanabe et al 1988a) and health issues (Roger and Bhuiyan 1990), little attention has been paid to the effects of agrochemicals and the rice crop itself on floodwater ecology. Studies of the impacts of factors associated with crop intensification in flooded rice soils, hitherto mostly restricted to short-term laboratory experiments, must be performed under more realistic field conditions and cultural practices over the long term.

# Agronomic management of the floodwater ecosystem

Agronomic management of the floodwater ecosystem includes a number of practices that affect the photosynthetic aquatic biomass (PAB) and the floodwater fauna. PAB management aims mostly at 1) controlling algal and aquatic macrophytic weeds, and 2) using  $N_2$ -fixing algae and *Azolla* as biofertilizers. Fauna management aims mostly at 1) controlling animals detrimental to rice, 2) controlling vectors of human and animal diseases, and 3) producing additional protein food in the field by raising edible animals.

## 7.1. Control of detrimental algae and aquatic macrophytes

This section is not intended to cover the whole topic of weed control in ricefields. It considers 1) the control of algal and submerged macrophytic weeds with algicides, 2) practices to control algae, which contribute to N losses by  $NH_3$  volatilization through their effects on floodwater chemistry, and 3) the biological control of weeds with aquatic organisms.

### 7.1.1. Algae and aquatic weed control

As reported in section 4.8, algae and aquatic macrophytes growing in the floodwater are not usually considered as major weeds in ricefields. However, when growing profusely—especially in direct seeded rice (DSR) at the beginning of the crop—they may significantly reduce yield. Literature on algae and submerged weed control in ricefields is scarce and there are few recent papers on this topic. In the last international conference dealing with weed control in rice (IRRI 1983b), almost no attention was paid to algae and submerged macrophytes, which probably indicates that they are only of local importance. Both algae and aquatic macrophytes can be controlled by algicides or physical methods.

7.1.1.1. *Algicides used in rice cultivation.* Copper-based algicides including  $CuSO_4$  and copper oxychloride are probably the most commonly used algicides. General aspects of Cu use as an algicide were reviewed by Gibson (1972). Copper use against algae in ricefields is documented in Argentina (Almazan and Robles 1956), India (Singh 1961, Mukherji and Laha 1979), Russia (Kayumov 1963), Italy (Bisiach 1971), the USA (Dunigan and Hill 1978), and Australia (Noble and Happey-Wood 1987).

Copper is generally used at 2-4 ppm; a concentration of 6 ppm can be used for direct application on floating algal masses. However an early application is more efficient.  $\text{CuSO}_4$  or copper chelates were successfully used for algal control in ricefields when applied before a bloom started, or at the first sign of a bloom (Dunigan et al 1979). The observation that young cultures of *Anabaena flos-aquae* were more susceptible to Cu than old ones (Gibson 1972) confirms a better efficiency of Cu-based algicides when applied before algal blooming.

Copper had no adverse effects on vegetative rice growth or grain yield (Dunigan et Hill 1978). Concentrations in seeds from the treated plots were slightly in excess of those in seeds from the control plots, but none of the values was considered high (Dunigan et al 1979). Copper does, however, have disadvantages in ricefields: 1) many species of algae are not controlled and Cu application might just change the nature of the dominant alga—an effect that was also observed in reservoirs (Gibson 1972), 2) Cu is expensive, 3) it is toxic to fish, and 4) it is rapidly inactivated by precipitation and adsorption on colloids, which may, over the long term, increase Cu concentration in soil to levels detrimental to rice. Although it is effective at low concentrations in large water bodies, it is not always effective in ricefields at rates up to 20 kg/ha (Misra et al 1976).

Batalla (1975) indicated that there were no algicides specially designed for use in ricefields but numerous pesticides, especially fungicides, have been tested for their algicidal potential on ricefield algae.

Several screenings were conducted in vitro. Minervini Ferrante et al (1974) used *Oscillatoria* and *Cylindrospermum* (cyanobacteria) growing on agar plates. The method was reported to be effective, giving prompt and reproducible results. Pesticide efficiency, however, is usually higher in vitro than in situ. Small-scale tests in the presence of soil are more representative (Bisiach 1971). Estimates of algicidal level of pentachlorophenol obtained in beakers with soil were similar to those determined in situ (Mukherji 1972).

Bisiach (1971, 1972) compared in vitro algicidal properties of Brestan (triphenyltin acetate), Benzuride (N-benzoyl-N-(3,4 dichlorophenyl)-NN'-dimethylurea), Captafol (N-(1,1,2,2, tetrachloroethylthio)-3a,4,7,7a tetrahydrophthalimide), HOE 2997 (2,dichloroacetamino-3, chloro,1,4 naphthoquinone), Folpet (N-(trichloromethylthio)phthalimide), and  $\text{KMnO}_4$  on a range of microalgae. All the chemicals except Benzuride showed a marked algicidal activity. HOE 2997 was a good substitute for the tin derivative (Brestan). It had a complete algicidal activity at 1-4 ppm against *Oedogonium*, *Hydrodictyon*, and *Spirogyra*, was not phytotoxic for rice, and had a very high  $\text{LD}_{50}$  for rat ( $1.5 \times 10^4$  mg/kg). Tests performed by Minervini Ferrante et al (1974) confirmed the high activity of the naphthoquinone derivative, an apparent alternative to tin compounds, which are highly toxic to vertebrates.

In addition to Cu-based compounds, Batalla (1975) listed five major groups of algicides.

- Dithiocarbamates (zineb, maneb, mancozeb, propineb, nabam, metiram) commercialized as fungicides that can be used as algicides at dosages of 8-20 kg ai/ha.
- Phthalimides or imide derivatives (Captan, Folpet, Captafol) are basically cryptogamicides. They can be used as algicides at 3-4 ppm ai. A higher concentration 10-15 ppm was suggested for submerged aquatic macrophytes.
- Naphthoquinone derivatives (dichlone, Chironamid) applied at 3-4 ppm ai. Chironamid was reported to have no significant effect on floodwater invertebrates.
- Organic salts of tin (Fentin) applied at 3-5 kg ai/ha. Their high toxicity to warm-blooded organisms restricts their use.
- Quaternary salts of ammonium (Dimanin, Hyamine) applied at high concentrations (10-40 ppm).

As a preventive measure, algicides can be applied on the seeds and in the water at the water inlet. For curative applications, the water level should be lowered before applying the algicide, and irrigation should be avoided for 48 h.

One major concern of algicide use is the potential toxicity to rice and edible animals growing in ricefields. In Louisiana, triazines applied at the level needed for controlling algae that can cause mechanical damage to young rice seedlings, *algal scum*, were toxic to rice (Dunigan et al 1979). In India, pentachlorophenol applied at 3-5 kg/ha was efficient in controlling macrophytic algae, but was toxic to fish and snails (Mukherji 1972). Quantitative data for field use of 18 algicides are found in Batalla (1975) together with toxicity levels for some freshwater fish.

7.1.1.2. *Algae and aquatic plants treated with algicides.* Algicides are applied on two major kinds of algal blooms in ricefields: those due to microalgae and those formed by submerged macrophytic algae or aquatic plants such as *Chara*. Both are considered detrimental when they develop at the beginning of the crop cycle (see sect. 4.8).

Blooms of unicellular algae are responsible for the so-called *slime* or *scum*, which may cause problems in DSR (Noble and Happey-Wood 1987) or young transplanted seedlings (Vasistha 1928). These blooms are most often controlled by Cu-based algicides (Vasistha 1928, Noble and Happey-Wood 1987). Di-(N, N-dimethylcocoamine) and mono (N, N-dimethyl alkylamine) formulations of endothal were the most effective for controlling filamentous green algae *Oedogonium* spp. and *Rhizoctonium* sp. Dichlobenil, a preemergence herbicide, controlled *Cladophora* and *Pithophora* spp. (Das 1976). Cyanobacteria are often more resistant to pesticides than eukaryotic algae (see sect. 6.3.3). That was also observed with algicides in Italian ricefields where *Anabaena* sp., *Nostoc* sp., and *Oscillatoria* sp. developed after application of Fentin derivatives and sodium dithiocarbamate against green algae (Bisiach 1971).

Microalgae can also be controlled by completely draining the affected field (Smith et al 1977, Noble and Happey-Wood 1987), but farmers are often reluctant to drain their fields because of wasting water. It was also observed that blooms of unicellular algae may result from the use of insecticides against *blood worms* (*Chironomus* spp.), which might damage rice roots but are also algal grazers. After insecticide use was discontinued, little slime problem was encountered (Noble and Happey-Wood 1987).

Blooms of macrophytic algae can develop very large biomasses. Therefore, their mechanical or manual control is not feasible because it is costly and injures the crop. Reinfestation often occurs during the same crop after manual weeding because of incomplete removal (Mukherji 1968).  $\text{CuSO}_4$  and copper oxychloride have been successfully used to control macrophytic algae *Chara* and *Nitella* (Mukherji and Sengupta 1964, Batalla 1975, Mukherji and Laha 1979). Naphthoquinone derivatives are especially efficient against blooms of Potamogetonaceae (Batalla 1975). In field trials, Mukherji (1972) observed a 90% kill of *Chara* and *Nitella* in ricefields using pentachlorophenol or sodium pentachlorophenate at 5 kg/ha. Sodium salts of 2, 4-D and dithiocarbamate were also efficient (Misra et al 1976). When Machete was used to control *Chara* and *Nitella*, rice yield increased by 47%; 26% with  $\text{CuSO}_4$ ; and 24% with Dithane. Lasso had no effect on algae or yield (Mukherji and Laha 1979).

### 7.1.2. Decreasing losses due to ammonia volatilization

Numerous studies have shown that N fertilizer recovery in wetland rice is seldom more than 30-40% and, even with the best agronomic practices and strictly controlled conditions, rarely exceeds 60-65% (De Datta 1981). In wetland soils, N is the *escape artist* among plant nutrients (Hauck 1980). It has the greatest potential for losses that occur through denitrification,  $\text{NH}_3$  volatilization, leaching, and runoff. Loss through denitrification is mostly a microbiological process. Loss through  $\text{NH}_3$  volatilization results from a chemical process caused mostly by a marked increase in floodwater pH in relation to algal activity.

Practices that decrease algal growth, such as the application of  $\text{CuSO}_4$  (Mikkelsen et al 1978) and other algicides (Bowmer and Muirhead 1987), and deep placement of N fertilizer (Zhi et al 1984) decrease diurnal pH variations and maximum value, and therefore N losses.

Preventing  $\text{NH}_3$  buildup in the floodwater after fertilizer application is another possible method to control algal growth. That too can be achieved by deep placement of fertilizer in the soil or by the inhibition of urease, which hydrolyzes urea to ammonium bicarbonate (Vlek et al 1980).

7.1.2.1. *Algicides*. Few reports describe the potential use of algicides to decrease N losses and improve fertilizer efficiency. Algicides currently being tested for their effect on  $\text{NH}_3$  volatilization are  $\text{CuSO}_4$ , simazine (2-chloro-4, 6-bis(ethylamino-s-triazine), diuron (3-(3,4-dichlorophenyl)-1,1 dimethyl-

urea), and terbutryne (2-(*tert*-butylamino)-4-(ethylamino)-6-(methylthio)-s-triazine).

Copper applied at 1.5 kg/ha with various levels of urea had little effect on algal growth and no effect on pH, O<sub>2</sub> content, or ammoniacal N content of the water (Muirhead et al 1990).

Simazine applied with urea (Vlek et al 1980), diuron applied with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (IRRI 1977), and terbutryne applied with urea (Bowmer and Muirhead 1987) reduced water pH and increased NH<sub>4</sub><sup>+</sup>-N concentration in the floodwater compared with the controls. Terbutryne applied at panicle initiation (PI) in a temperate ricefield decreased water pH for 6 d and N losses by 43%, but the resulting 5% increase in yield was too small to be statistically detectable (Bowmer and Muirhead 1987). Terbutryne applied with urea 10 d after transplanting (DT) and at PI in a tropical ricefield reduced the maximum floodwater pH by 0.9 unit in the dry season (DS) and by 0.5 unit in the wet season (WS) for more than 1 wk. The estimated saving averaged 4.7 kg N/ha when 90 kg N/ha was applied, and 9.6 kg N/ha when 150 kg N/ha was applied (Muirhead et al 1990).

Bowmer and Muirhead (1987) pointed out that by reducing photosynthetic O<sub>2</sub> production at the soil surface, algicides might also reduce nitrification and thus the potential for losses by denitrification.

Available data suggest that fertilizer saving from algicide use is low. More data are needed for definite conclusions, but deep placement of N fertilizers, which prevents the rapid growth of non-N<sub>2</sub>-fixing algae and prevents high concentration of NH<sub>3</sub> in floodwater, seems a more efficient method to decrease N losses by NH<sub>3</sub> volatilization and increase fertilizer efficiency (Roger et al 1980, Simpson et al 1994a).

Measurements at IRRI showed that submerged macrophytes (*Chara*, *Najas*, etc.) significantly increased floodwater pH, whereas it was fairly stable under floating macrophytes such as *Azolla* and *Lemna*. That indicates a potential for combined use of *Azolla* and chemical N.

7.1.2.2. *Urease inhibitors*. Another possible method for preventing the buildup of NH<sub>3</sub> in the floodwater following fertilizer application is the inhibition of urease, which hydrolyzes urea to ammonium bicarbonate (Vlek et al 1980). Urease inhibitors might offer the attractive possibility of reducing N loss through a modified fertilizer formulation, without requiring a change in the farmer's application practice (De Datta and Buresh 1989). Very few of the chemicals tested, however, met the requirements of an effective urease inhibitor for use with urea fertilizer (Hauck 1984). Phenyl-phosphoro-diamidate (PPD) and N-(*n*-butyl) thiophosphoric triamide (NBPT) were tested for rice.

PPD decreased NH<sub>3</sub> evolution from some soils in the laboratory. Results of field trials were negative (Hauck 1984), which was attributed to a fast degradation of PPD by soil microorganisms. Hauck concluded that no substance patented or under study could be recommended for commercial production at that time. In further studies, PPD decreased N losses by volatilization (Fillery

and De Datta 1986) but did not consistently increase yield (Fillery et al 1986). Both PPD and NBPT retarded the disappearance of urea from floodwater. But whereas PPD only delayed the buildup of  $\text{NH}_3$  in the water, NBPT prevented it, reducing partial pressure on  $\text{NH}_3$  in floodwater by an average 80-98% during the 10 d following urea application. Significant yield increase (average 8%) was observed at only one of the two sites tested (Buresh et al 1988a,b).

In a 2-yr  $^{15}\text{N}$  balance study of multiple urea rates in the Philippines, Buresh et al (1988c) estimated the maximum potential of urease inhibitors. They considered unaccounted  $^{15}\text{N}$ , in the absence of leaching and runoff, as the potential saving when urea N is used with an ideal inhibitor — one that would completely eliminate gaseous losses without detrimental effect on rice. They concluded that such an ideal urease inhibitor could increase yield by a maximum of 6-8%.

Urease inhibitors, although they reduce the buildup of ammoniacal N after urea application, also tend to delay the disappearance of urea from floodwater, which results in a higher total N (urea +  $\text{NH}_4^+$ ) concentration in floodwater (De Datta and Buresh 1989). That may increase loss by runoff when water flows out of the fields soon after fertilizer application.

### 7.1.3. Biological control of weeds

Biological control of weeds is not a recognized weed management tool, but some practices have shown a potential. Among them are

- *Azolla* inoculation in ricefields, which may control certain weeds by competition,
- stocking invertebrates that disturb the upper soil layer and feed on seeds and young roots of weeds, and
- stocking herbivorous fish that feed on weeds.

7.1.3.1. *Weed control with Azolla*. Several reports indicate an overall reduction of weed incidence in plots inoculated with *Azolla* (Lumpkin and Plucknett 1982, Diara et al 1987, Kannaiyan 1987). But weeds differ in their response to *Azolla* competition. Using three fields with different weed flora, Janiya and Moody (1981b) found that *Monochoria vaginalis*, *Echinochloa glabrescens*, *Cyperus difformis*, and *Paspalum* sp. were controlled with a degree of suppression ranging from 70 to 100%. *Azolla* failed to suppress the growth of *Scirpus maritimus* and *Echinochloa crus-galli*.

7.1.3.2. *Weed control with fauna*. The crustaceans *Triopus longicaudatus*, *T. granaris*, and *T. cancrifonnis* (tadpole shrimp) have the potential for biological control of weeds because they feed on young seedlings and disturb their roots by mechanical agitation of the soil. Their potential was studied by Matsunaka (1976, 1979) in Japan. Populations of 20-30 tadpole shrimp/m<sup>2</sup> reduced weed populations in farmers' fields and labor for weeding by 70-80%. Eggs of the shrimp tolerate environmental changes and are more suitable than adults for stocking in ricefields. Eggs applied after flooding yielded larger populations

than those applied 3-9 d before flooding or than adults applied to the floodwater.

This technique is limited to transplanted rice because tadpole shrimp are not selective and may also damage the roots of germinating rice and young seedlings. Both immature forms and adults are sensitive to insecticides and certain herbicides, thus the timing of chemical applications is critical to the successful use of the crustacean.

Introduction of herbivorous fish into ricefields provides some weed control. That is covered together with rice-fish culture in sect. 7.6.4.3.

## 7.2. Use of aquatic macrophytes as organic fertilizer

Algae and aquatic weeds that develop in ricefields and their related bodies of water constitute a source of organic matter (OM) and nutrients that can be used as manure for rice. Standing crops of PAB often attain 500 kg dry weight (dw)/ha in the fields and 1-5 t dw/ha in irrigation canals and water tanks. Higher values (30 t dw/ha) have been reported in bodies of water receiving farm or factory effluents. Planktonic algae usually have a lower productivity than aquatic macrophytes.

The average composition of aquatic macrophytes is 8% dry matter (DM), 2-3% N (dw basis), 0.2-0.3% P, and 2-3% K. Planktonic algae have higher N contents, averaging 5%. On a dw basis, this composition is very similar to that of many green manures except for K in macrophytes and N in planktonic algae, which are higher. However, the high water content of aquatic weeds has been the major deterrent to their use.

Shortages of food and fertilizers and large expanses of aquatic weeds often exist in the same locality (Boyd 1974). The use of aquatic weeds from water bodies as a manure is an integrated management strategy that permits both the reclamation of the water body and the fertilization of a crop with an organic manure frequently rich in N, P, and K. Moreover, aquatic plants can possibly be used to remove nutrients from wastewater effluents. Pilot studies with *Eichhornia* indicated that up to 29 t dw/ha can be produced in ponds receiving additional nutrients (Wahlquist 1972).

### 7.2.1. Potential as organic manure and current use

Because their N, P, and K contents are similar to those of many green manures used in dryland soil, partially dried, composted, aquatic plants are a suitable soil fertilizer and conditioner. Several aquatic weeds have been used to make compost, mulches, and fertilizers; Little (1968) compiled a variety of methods. The same author (1979) recorded 26 papers dealing with the topic, 19 of which dealt with water hyacinth but only 3 with its use in ricefields, clearly demonstrating the emphasis given to water hyacinth and dryland soils. The Indian Council of Scientific and Industrial Research (1952, cited by Little 1979)

indicated that water hyacinth compost was eminently suitable for jute and ricefields. Subagyo and Vuong (1975, cited by Little 1979) reported that dead masses of *Salvinia molesta* stimulated the growth of rice seedlings in Indonesia.

Majid et al (1980) reported the effects of drained algae, composted aquatic weeds, and cow manure on a variety of crops including rice, soybean, sesame, brinjal, garlic, and onion. Drained algae and composted aquatic weeds were as good as or better than cow manure. Field experiments indicated that composted *Eichhornia*, in addition to the usual dose of chemical fertilizer, increased rice yield by 24%.

Submerged weeds and filamentous green algae that grow in irrigation canals can be incorporated into soil as a source of OM for rice. *Cladophora* and *Spirogyra*, two filamentous freshwater algae, were used by Pantastico and Rubio (1971) as manure for rice. A higher yield increase was obtained with dried algae than with fresh algae. *Spirogyra*, richer in N (5% dw), was more efficient than *Cladophora* (N = 2.39% dw). For an equivalent quantity of N,  $(\text{NH}_4)_2\text{SO}_4$  was more efficient than dried algal material.

Little is known about the effect of incorporation of weeds or weed compost on soil properties. Dhar (1961) reported that incorporation of water hyacinth into soil resulted in a higher  $\text{N}_2$ -fixing activity when basic slag (0.5%  $\text{P}_2\text{O}_5$ ) was added and when the soil was exposed to light. Depending on the treatment,  $\text{N}_2$ -fixation efficiency ranged from 18.2 to 33.5 mg N/g oxidized C.

Aquatic weeds (mainly water hyacinth and *Pistia*), growing naturally or cultivated, have been used as organic manure for wetland rice in China (Nan Kin Institute of Soil Science 1978). In Hunan Province, aquatic plants, mainly *Pistia stratiotes*, *Eichhornia crassipes*, and *Alternanthera sessilis*, were collected or grown for making compost or pig food (IRRI 1980). In Fujian Province, China, yield trials were conducted with a wheat - rice - rice rotation (wheat followed by two rice crops) using organic manures. Use of 75 t mud manure/ha per year gave yields between 7.5 and 11.8 t/ha per crop. Mud manure is prepared by mixing mud with aquatic plants (mainly *Eichhornia*), flooding for a while to permit anaerobic decomposition, and then draining to permit aerobic incubation. In the same area, trials to grow aquatic plants (*Pistia stratiotes*, *Japonica narcissus*, and *Azolla pinnata*) within wide rows of late rice were conducted. Chinese scientists indicated that non- $\text{N}_2$ -fixing aquatic weeds collected N from floodwater, and the accumulated N was incorporated into the soil.

*Salvinia molesta* has been widely used by farmers in West Java, Indonesia, as a soil additive in ricefields; 40 t/ha increased rice tillering by 30%. *Eichhornia crassipes* has also been used after being composted for 3-4 mo (Soerjani 1980).

Thus, it appears that aquatic plants have been used as a source of OM and nutrients mainly in dryland soils, and that their potentials on wetland soils are poorly documented, except in China.

### 7.2.2. Limiting factors

There are limitations to and possible noxious effects from the use of algae and aquatic macrophytes. One limitation is the bulkiness of the material, despite the fact that in most places where tropical water weeds are a problem there is ample solar radiation to dry the harvest. Little and Henson (1967) pointed out that the harvest of water weeds is commonly believed to be too labor-intensive because of their high water content (average 92%). To obtain the same DM of plant material from water weeds (8% dw), about twice as much fresh material is needed as lucerne (15% dw) or 2.5 times that of pasture grasses (20% dw).

A second possible limitation on the use of aquatic weeds in wetland soils is that they can aid weed dispersal through the irrigation system to the ricefields. Gupta (undated) pointed out that many weed cuttings and rhizomes regain viability even after composting.

A third limitation is that fresh algae and aquatic weeds can release products toxic to rice when incorporated. Composting, therefore, is recommended as a precautionary measure. Buried aquatic plants decompose and liberate organic acids that are toxic to rice and create an unfavorable pH (Kotalawa 1976). Mats of cyanobacteria incubated anaerobically rapidly produced large amounts of volatile S compounds, including H<sub>2</sub>S, methyl mercaptan, and dimethyl sulfide (Zinder et al 1977).

A fourth limitation is that pesticides, industrial chemicals, and heavy metals may be absorbed and concentrated by aquatic plants (Vance and Drummond 1969, Rose and McIntire 1970). Water hyacinths, in particular, absorb large quantities of toxic materials, including heavy metals. Some filamentous algae appear to be especially efficient in accumulating pesticides, e.g., *Cladophora* concentrates DDT far more than other plants or animals (Meeks and Peterle 1967).

Incorporating OM from aquatic weeds then is not invariably beneficial. Singh (1962) compared the effects of composts made from a number of aquatic weeds on the yield of different fruits. With composts of *Pistia*, *Najas*, *Hydrilla*, and *Ottelia*, yields were higher than in the control, but with *Eichhornia* they were consistently lower.

Algae and weeds growing in irrigation canals and in other bodies of water may have very high productivity and are, in most cases, considered detrimental. Using them as a source of compost can effect the reclamation of bodies of water. Such use has been developed mainly for dryland crops and, except in China, little is known about its potential for wetland rice. The positive effects on grain yield have been reported, but detrimental effects such as weed dispersal and concentration of pesticides and heavy metals are possible. Therefore, discretion should be exercised when considering use of aquatic weeds in wetland soils. Moreover, because of the bulkiness of aquatic weeds, their use will be strongly influenced by agro-economic conditions, the ease of harvest, and the distance from the field to where they are composted.

### 7.3. Use of *Azolla* as green manure

Cyanobacteria symbiotic associations with plants exist with one or a few genera of phycmycetes, ascomycetes, green algae, diatoms, liverworts, mosses, ferns, gymnosperms, and angiosperms. The only symbiosis of agronomic significance is that between *Anabaena azollae* and aquatic ferns of the genus *Azolla*. There are seven species of *Azolla* in two extant sections (Table 7.1), taxonomically separated by their secondary reproductive structures. Several species are also distinguishable by the branching patterns or growth habits of their sporophytes. The endophytic *Anabaena azollae* resides within a basal cavity of the dorsal lobe of each leaf of *Azolla*, but its cells are also associated with the terminal meristem and the megasporocarp. Filaments of the cyanobacterium have heterocyst frequencies of 20-30% when actively fixing N<sub>2</sub> in the leaf cavities (Hill 1975). By inducing N excretion by the cyanobacterium through the repression of its glutamine synthetase levels, the N needs of *Azolla* are also met (Ray et al 1978). The entire association may proliferate without mineral N.

Because of its rapid growth, its ability to grow together with rice in submerged soils, and its high N content, *Azolla* has been used as a green manure in rice culture for centuries in northern Vietnam and southern China. Its use was generally ignored by scientists in other countries until the mid-1970s. Since then, articles, reviews, and information gathered from China and Vietnam have stimulated interest in and research on *Azolla* (Watanabe 1982). *Azolla* use was reviewed by Lumpkin and Plucknett (1982). Proceedings of two workshops on *Azolla* (IRRI 1987b, Silver and Shröder 1984) provide additional information. Two issues of *The Philippine Agriculturist* (1986a,b) were devoted to free-living and symbiotic cyanobacteria in the Philippines, where a National *Azolla* Action Program was established in 1982.

**Table 7.1. Taxonomy and continental range of extant *Azolla* species (Roger et al 1993).**

Species	Indigenous distribution
Section <i>Azolla</i>	
<i>A. filiculoides</i> Lamarck	North and South America
<i>A. mexicana</i> Presl	North and South America
<i>A. microphylla</i> Kaulfuss	North and South America
<i>A. caroliniana</i> Willdenow	North and South America
<i>A. rubra</i> R. Brown	Asia, Australia
Section <i>Rhizosperma</i>	
<i>A. pinnata</i> R. Brown	
var. <i>pinnata</i> R. Brown	Australia, Africa
var. <i>imbricata</i> Roxburgh	Asia
<i>A. nilotica</i> DeCaisne	Africa

### 7.3.1. Ecology of *Azolla* in ricefields

7.3.1.1. *Occurrence of Azolla in ricefields.* A review of the phytogeography of *Azolla* species (Lumpkin and Plucknett 1982) shows that *Azolla* is widely distributed throughout the world, occurring in a broad latitudinal range on five continents. The international bibliography on *Azolla* (Capaya 1979, IRRI 1983a) cites almost 50 articles recording *Azolla* as an aquatic weed in many rice-growing countries. To develop significant biomass in ricefields, however, the fields need to be inoculated with *Azolla* and the *Azolla* cultivated.

7.3.1.2. *Climatic factors.* The optimum temperature for most species (20-30 °C) is below the average temperature in the tropics. Cool weather is a key to successful *Azolla* cultivation in Vietnam and China. Some strains, however, can grow at temperatures higher than 35 °C (Lumpkin 1987, Watanabe and Berja 1983). The successful growth of *Azolla pinnata* at high temperature observed in north Senegal, where humidity is low (Diara et al 1987), indicates that in the humid tropics the detrimental effect of high temperature might be mostly indirect, resulting in a high incidence of insect and fungal pests.

Light saturation for *Azolla* growth was reported to occur at 20-50% of full sunlight (Ashton 1974, Peters et al 1980), and optimum growth and N<sub>2</sub>-fixing activity were observed at about 50% of full sunlight (Li ZuoXin et al 1987). According to Lumpkin, *Azolla* is not inhibited by full sunlight as long as other factors are not limiting (Lumpkin 1987, Lumpkin and Plucknett 1982). On the other hand, several reports indicate a decrease in growth or N<sub>2</sub> fixation at various light intensities between 60 and 120 klx (Ashton 1974, Becking 1979, Li ZuoXin et al 1987, Roger and Reynaud 1979b). When *Azolla* is grown with rice, shading by the rice starts decreasing *Azolla* growth 2-3 wk after transplanting and inhibits it after about 45 d (Lumpkin 1987). Daylength is correlated with *Azolla* growth (Lumpkin and Bartholomew 1986), which indicates better potential for *Azolla* cultivation in higher latitudes with long, cool days than in the tropics with constant daylength (Lumpkin 1987).

7.3.1.3. *Physicochemical factors.* The pH for optimum growth of *Azolla* in culture solution is in a range of 4.5-7.0, but *Azolla* can survive within a range of 3.5-10 (Lumpkin 1987). The major effect of pH on *Azolla* growth is probably related to nutrient deficiencies (Fe, Zn, etc.) occurring at high values or toxicities (Al, Fe, etc.) occurring at low values (Watanabe et al 1977a).

Phosphorus is most frequently the major limiting nutrient for *Azolla* growth in the field. Reported threshold values of P deficiency are 0.4% in *Azolla* (dw basis), 0.15 ppm in floodwater, and 20 ppm available Olsen P in soil (Ali and Watanabe 1986, Watanabe and Ramirez 1984). *Azolla pinnata* can grow satisfactorily without P application when the available Olsen P in the soil is higher than 30 ppm and P sorption capacity is lower than 660 mg P/100 g (Watanabe and Ramirez 1984). Such P-rich soils are not very common. *Azolla* doubling time estimated in a growth test on 972 Philippine soils was less than 5 d (moderately suitable soil) in 40% of the samples and less than 3.5 d (highly suitable) in only 13% of the samples, showing that P fertilization of

*Azolla* would be required in many soils (Callo et al 1985). That was confirmed by the observation that 80% of field-grown *Azolla* has a P content below 0.4% and a N content below 4%.

Other nutrients seem to be much less limiting in situ than P. In the Philippines, total C, total N, and exchangeable cations of soils were not correlated with *Azolla* productivity (Watanabe and Ramirez 1984). Potassium, however, was necessary for *Azolla* growth in northern Vietnam (Roger and Watanabe 1986). Lumpkin (1987) cites four references reporting that the addition of Mo or Fe improved *Azolla* growth in the field.

7.3.1.4. *Biotic factors.* Grazing by invertebrates is one of the factors limiting *Azolla* growth. The *Azolla* mat floating on the floodwater constitutes an environment that favors the development of invertebrate populations, including insect larvae and molluscs that feed on *Azolla*. A survey of the invertebrates associated with *Azolla* at several sites in the Philippines identified 27 species, among which 6 insects and 2 snails were found detrimental to *Azolla* (Calilung and Lit 1987). Yield losses of field-grown *Azolla* due to *Ephestiopsis vishnu* (webworm) and *Nymphula* spp. (caseworms) ranged from 6 to 64% for *Azolla microphylla* and from 13 to 57% for *A. pinnata* (Mochida et al 1985). A tentative list of *Azolla* pests includes 30 insects (13 Diptera, 6 Coleoptera, 8 Lepidoptera, 1 Homoptera, 2 Orthoptera), 2 Arachnoidea, and 9 Mollusca (Mochida 1987).

Some fungi are *Azolla* pathogens (Arunianart et al 1982, Garcia 1986, Shahjahan et al 1980). These fungi magnify the effects of insect attacks on *Azolla* because they develop on the plant wounds (Lumpkin 1987). *Azolla* resistance to fungal pathogens is associated with the strain's ability to grow under low light intensities (Liu 1987).

A negative correlation has been observed between *Azolla* and algal biomass in ricefields at IRRI (P. A. Roger, S. S. Ardales, and R. R. Jimenez, IRRI, 1989, unpubl.). Algal blooms may compete with *Azolla* for nutrients and, by increasing floodwater pH, may reduce nutrient availability to *Azolla*.

7.3.1.5. *Effect of N fertilizer.* In the absence of competing organisms, N<sub>2</sub> fixation by *Azolla* is more tolerant of combined N than N<sub>2</sub> fixation by free-living organisms. When *Azolla caroliniana* was grown in nutrient solutions of NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, and urea, all at 35 ppm N concentration, it derived 86% of its N from biological N<sub>2</sub> fixation (BNF) in NO<sub>3</sub><sup>-</sup> solution, 70% in NH<sub>4</sub><sup>+</sup> solution, and 60% in urea solution (Peters et al 1981). Ten mM urea N or NH<sub>4</sub><sup>+</sup>-N and 25 mM of NO<sub>3</sub><sup>-</sup>-N reduced the acetylene-reducing activity (ARA) of *Azolla* by about one-half, whereas the ARA of free-living cyanobacteria was completely repressed after 24 h of exposure to 1 mM NH<sub>4</sub><sup>+</sup> (Ito et al 1983). In floodwater, however, N favors the growth of competing aquatic plants that may hinder *Azolla* growth. In the presence of green algae, 1.4 mM NH<sub>4</sub><sup>+</sup>-N decreased *A. pinnata* growth by 60% (Watanabe et al 1977a).

The *Azolla* canopy prevents light penetration into the floodwater, inhibits the growth of other phototrophs, and depresses photodependent CO<sub>2</sub> up-

take. Thus, under an *Azolla* canopy, the floodwater pH remains lower than in *Azolla*-free conditions. The presence of *Azolla* may therefore be expected to decrease N losses by NH<sub>3</sub> volatilization.

7.3.1.6. *Effect of pesticides.* Herbicides have been shown to be toxic to *Azolla* even though one of the recognized beneficial effects of *Azolla* is its weedicide effect. Therefore there is little interest in combining the use of *Azolla* with that of herbicides. Threshold values for toxicity are much higher for insecticides than for herbicides. Insecticides favor *Azolla* growth by decreasing insect pest incidence, but they may also be phytotoxic (see sect. 6.3.3).

### 7.3.2. Estimation of the nitrogen potential of *Azolla* in ricefields

The potential of *Azolla* for rice production was summarized by Roger and Watanabe (1986) who considered 1) growth rate, 2) maximum biomass, 3) N<sub>2</sub>-fixing activity, and 4) experiments comparing *Azolla* use with mineral fertilizer.

Doubling time varied between 2 and 10 d for most species.

The reported maximum standing biomass ranged from 0.8 to 5.2 t DM/ha and averaged 2.1 t/ha (n = 13; CV = 57%). The corresponding N content ranged from 20 to 146 kg/ha and averaged 70 kg/ha (n = 17; CV = 58%).

The N<sub>2</sub>-fixing rate ranged from 0.4 to 3.6 kg N/ha per day and averaged 2 kg N/ha per day (n = 15, CV = 47%) (Kikuchi et al 1984). In 1 yr, a continuous *Azolla* culture, repeatedly harvested, can fix from 500 kg N/ha (*Azolla pinnata*) (Watanabe et al 1980) to 1,200 kg N/ha (*A. filiculoides*) (Li ZuoXin et al 1982). When dual-cropped with rice, *Azolla* can accumulate from 25 to 170 kg N/ha in 60 d (Kikuchi et al 1984), which may exceed the N requirement of rice. *Azolla* N<sub>2</sub>-fixing activity per unit area is similar to that of legume pastures or even greater. International field trials conducted for 4 yr at 37 sites in 10 countries (Watanabe 1987) showed that 1) incorporating one crop of *Azolla* grown before or after transplanting is equivalent to application of 30 kg fertilizer N/ha, and 2) incorporating two *Azolla* crops grown before and after transplanting is equivalent to split application of 60 kg N/ha (Table 7.2).

### 7.3.3. Effects of *Azolla* on rice production

7.3.3.1. *Use of *Azolla* nitrogen by rice.* Nitrogen recovered by the rice plant from <sup>15</sup>N-labeled *Azolla* incorporated into the soil ranged from 20 to 34% (Table 4.10). Methods of application were compared by Ito and Watanabe (1985). As with cyanobacteria, availability decreased when *Azolla* was surface-applied. When *Azolla* was placed on the soil surface, about 66% of its N was lost and 12-14% was recovered by the rice. When *Azolla* was incorporated, losses were reduced and availability increased to 26%.

7.3.3.2. *Other effects beneficial to rice.* *Azolla* not only provides additional P for the rice crop but also enhances the use of P from mineral fertilizer (Sampaio et al 1984). The threshold concentration of K for absorption was estimated as 0.8.5 ppm K<sub>2</sub>O for *Azolla* and 8 ppm for rice (Liu 1984). Irrigation water usu-

**Table 7.2. *Azolla* trials at 37 sites in 10 countries by the International Network on Soil Fertility and Fertilizer Efficiency on Rice (Watanabe 1987).<sup>a</sup>**

Treatment	Yield (t/ha)	Index (%)
<i>1979-80 trials</i>		
1 Control, no N, no <i>Azolla</i>	3.00	100 c
2 30 kg N/ha, 3 split applications	3.65	121 b
3 60 kg N/ha, 3 split applications	4.24	141 a
4 <i>Azolla</i> incorporated before transplanting	3.73	124 b
5 <i>Azolla</i> incorporated after transplanting	3.67	122 b
6 <i>Azolla</i> inoculated after transplanting but not incorporated	3.61	120 b
7 Combination of treatments 2 and 4	4.15	138 a
8 Combination of treatments 2 and 5	4.07	135 a
9 <i>Azolla</i> incorporated before and after transplanting	4.09	136 a
Standard error between sites	0.05	
Standard within sites <sup>b</sup>	0.05	
<i>1982-82 trials</i>		
1 Control, no N, no <i>Azolla</i> (20 x 20)	2.94	100 c
2 60 kg N/ha, 3 split applications (20 x 20)	4.17	141 a
3 60 kg N/ha, 3 split applications (10 x 40)	4.20	142 a
4 20 t <i>Azolla</i> /ha incorporated before transplanting + 60 kg N/ha, 3 split applications (20 x 20)	4.18	142 a
5 20 t <i>Azolla</i> /ha incorporated before transplanting + 60 kg N/ha, 3 split applications (10 x 40)	4.16	141 a
6 <i>Azolla</i> grown before and after transplanting and incorporated after full cover (20 x 20)	3.96	134 ab
7 <i>Azolla</i> grown before and after transplanting and incorporated after full cover (10 x 40)	4.04	137 ab
8 <i>Azolla</i> grown twice only after transplanting (10 x 40)	3.88	132 b
Standard error among sites	0.077	
Standard error within sites	0.052	

<sup>a</sup> In a column, means followed by a common letter are not significantly different at the 5% level by DMRT. <sup>b</sup> Values in parentheses are rice plant spacing in cm.

ally contains 1-5 ppm K, which is below the level at which rice can absorb K rapidly, but enough for rapid absorption and concentration by *Azolla*, which becomes a source of K for rice when incorporated (Liu 1984). Several reports note that *Azolla* decreased weed incidence (Diara et al 1987, Lumpkin and Plucknett 1982). The reduction in weed biomass at 40 DT (47-76%) was positively correlated with the quantity of inoculum applied 7 DT (1-5 t fw/ha) (Kannaiyan 1987). In Senegal, the *Azolla* cover decreased water evaporation

by 20% (Diara and Van Hove 1984). *Azolla* improves the structure of the soil as do most organic manures (Roychoudhury et al 1979,1983). That becomes important in fields where rice is grown sequentially with an upland crop.

### 7.3.4. Use of *Azolla* in rice cultivation

7.3.4.1. *Traditional methods.* China and Vietnam are the only countries with long histories of *Azolla* cultivation, extending back to the 11th century in Vietnam and at least to the Ming dynasty (1368-1644) in China (Lumpkin and Plucknett 1982).

In China, *Azolla* has been used from 37° N (Shandong) to 19° N (Hainan). Because the strains used grow best at an average daily temperature of 25 °C or below, *Azolla* is usually grown in late spring (May to June) in the north and in early spring (March to April) in the south. During summer, insect damage limits *Azolla* growth. Most frequently, *Azolla* is grown for about 1 mo, then incorporated before transplanting. To a lesser extent, wide-row transplanting permits cultivation of *Azolla* with rice and several incorporations during the crop cycle. In some places, *Azolla* is grown before and after transplanting (Liu 1979).

In Vietnam, *Azolla* is used in the northern provinces for spring rice because in summer it grows poorly and insect incidence is severe. About 2-3 mo before transplanting (August), *Azolla* is collected from natural environments by specialists and is multiplied on government *Azolla* farms. Inoculum is sold to cooperatives and farmers who propagate it in the fields from November to February. As in China, *Azolla* is grown and incorporated before and after transplanting. *Azolla* grown before transplanting is fertilized with 2.2 kg P/ha every 5 d, 4.2 kg K/ha every 10 d, and 0.5-1.0 t farmyard manure (FYM)/ha every 5-10 d. When chemical fertilizer is not available, ash is substituted. Intercropped *Azolla* is usually not fertilized, but if P is available, one application of 4.4 kg P/ha is recommended. *Azolla* inoculum is applied in the field at 0.3-0.5 t fw/ha along with MM. To facilitate vegetative multiplication, fronds are broken using a special tool. *Azolla* is collected before heavy rains to prevent its being washed from the fields. If insect pests develop, *Azolla* is collected and placed under water for 15 h to kill the larvae. Two to 3 wk after inoculation, the field is covered with about 20 t *Azolla* fw/ha. Half of this is collected in mounds and composted by covering it with soil. The remaining half is grown for 7-10 d more, after which the field is again fully covered. Half of the second crop and the compost are incorporated into the soil. Rice is transplanted and the remaining half of the second *Azolla* crop continues to grow. Seven to 10 DT, the field is covered, and half of the third crop is incorporated by hand and foot between the rows. Sometimes a fourth crop is grown and incorporated. This technology produces an average of 40 t *Azolla* fw/ha per rice crop, equivalent to 80 kg N/ha. The average annual rice yield in the Red River Delta is 5-7 t/ha. The winter crop, with *Azolla*, yields 3-5 t/ha, and the summer crop, without *Azolla*, yields 2 t/ha or less (Roger and Watanabe 1986).

The *Azolla* technology used in Vietnam is labor-intensive; it might be improved by wide-row transplanting of rice for easier *Azolla* incorporation with a rotary weeder.

7.3.4.2. *Integrated management of Azolla.* *Azolla* can also be used as feed for pigs, chickens, and fish. A rice - *Azolla* - fish system was developed in China. In field trials, average rice yield increased by 5% over the traditional system and an average fish production of 625 kg/ha was reported (Liu 1988). About 60% of the N<sub>2</sub> fixed by *Azolla* was assimilated by fish and 40% of this was excreted in feces equivalent to about 300 kg (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>/ha (Liu 1987). Weed biomass and the incidence of pests on rice and *Azolla* decreased (Liu 1988).

7.3.4.3. *Current use of Azolla.* Data concerning the extent of *Azolla* use in China are controversial. In 1978, FAO (1978) estimated that *Azolla* was used on more than 6.5 million ha in China. In 1979, Liu reported an estimate of 1.34 million ha. In 1982, Lumpkin and Plucknett (1982) reported that *Azolla* was used in 2% of the 34 million ha planted to rice in China.

In Vietnam, in 1980, *Azolla* was used in the northern and north central provinces on about 500,000 ha or 9% of the total rice area (Roger and Watanabe 1986).

In the Philippines, farmers adopted *Azolla* on 5,000 ha in South Cotabato in 1981 (Kikuchi et al 1984); success was due mainly to a high level of available P in the soils and a short DS. *Azolla* use extended to 26,000 ha in 1983, and 84,000 ha in 1985 (Mabbayad 1987). In the proceedings of the 1985 *Azolla* Workshop (IRRI 1987b), quantitative information on the rice area where *Azolla* is used is available only for the Philippines (84,000 ha). The papers presented during the workshop indicate that China, Philippines, and Vietnam were using *Azolla*. Brazil, India, Pakistan, Senegal, Sri Lanka, and Thailand were studying the feasibility of *Azolla* adoption by rice farmers.

With the advent of available, cheap sources of urea and potash, however, the area devoted to *Azolla* technology in Vietnam and China decreased dramatically during the 1980s. *Azolla* use has not spread to other countries. In Fujian Province, China, *Azolla* use has dropped to 5-10% of the rice hectareage, down from a high of 20% at the beginning of the 1980s. The same trend has been observed for green manures such as *Astragalus*, currently used on 10-20% of the land, down from a maximum of about 30% (Stone 1990). Interest in *Azolla* use as a fish and animal feed, a mineral scavenger, and depollutant, however, has increased in China (Liu 1984,1987). The reduction of *Azolla* use in China also stems from the changing governmental economic policy, which has led to the disbanding of many agricultural communes and the reallocation of labor. World *Azolla* use is now only a fraction of the estimated 2 million hectares of rice that were fertilized with *Azolla* in China and Vietnam in the late 1970s (Dao and Tran 1979, Liu 1979, IRRI 1980, Roger and Watanabe 1986).

7.3.4.4. *Limiting factors for Azolla use and possible remedies.* **Water control and maintenance of inoculum.** *Azolla* cannot withstand desiccation and requires water in the field throughout its cultivation cycle. Because *Azolla* is

propagated vegetatively, inoculum must be maintained in nurseries year-round and multiplied for distribution before field cultivation. This implies that an irrigation network and a network for inoculum conservation, production, and distribution are requisites for *Azolla* use. Further, *Azolla* adoption by farmers depends first on a government policy to establish such networks (Roger and Watanabe 1986).

*Azolla* sporocarps are more tolerant of adverse conditions than are sporophytes. A method for using sporocarps for inoculum conservation was developed in China, but the growth of sporocarps was too slow to meet inoculum requirement in the field; 160 kg sporocarps fw/ha yielded 16-21 t *Azolla* fw/ha in 52 d (Lu 1987). Conditions for sporocarp formation and germination are incompletely understood. Low temperatures stimulated sporulation in the tropics (Kannaiyan and Rains 1985). Germination of spores of *Azolla filiculoides* required 1) mixed cultivation of mature megasporocarps and microsporocarps, 2) sunlight, and 3) an average daily temperature of 20-30 °C. Higher temperatures within that range shortened the germination period (Xiao et al 1987).

**Need for P fertilizer.** As already stressed, P application is required in most soils for growing *Azolla*. To be economically feasible, P fertilization requires a ratio of N fixed to P applied that is greater than the ratio of the prices of the corresponding fertilizers. Basal application of P might have a low efficiency and be uneconomical. Yet split P application to increase *Azolla* growth could be economical, as shown by efficiency measurements of 4.6-10.0 g N<sub>2</sub> fixed/g P applied (Watanabe et al 1980,1988b). Phosphorus fertilization limited to the inoculum production plot permits the P-enriched *Azolla* to multiply 6 to 7 times without P application in the main field, and ensures a very high efficiency of applied P (Watanabe et al 1988b).

**Grazing.** Although commercial pesticides effectively control *Azolla* pests, no method of field application is economical (IRRI 1986). The possible use of commercial insecticides is limited to inoculum production (Mochida 1987). The use of cheap pesticides of plant origin (botanicals) in the field might be economical. Drainage, alternate drainage and irrigation, cultivation in a wet field or a thin layer of water, and reduced application of organic manure, especially N-rich manure, may help suppress the emergence and development of pests (Zhang et al 1987). Varietal differences in resistance to grazing by *Radix swinhoer* were reported (Liu 1984).

**Low temperature requirement.** Temperature limitations can be reduced by selecting cold- or heat-tolerant strains (Watanabe and Berja 1983). Among strains so far tested at IRRI, *Azolla microphylla* #418 was most tolerant of high temperature (37 °C d/29 °C night) (Watanabe et al 1992).

**Economics.** Technologies used in Vietnam and China are labor-intensive and therefore have economic limitations. Kikuchi et al (1984) studied the economics of *Azolla* use in the Philippines in South Cotabato, where *Azolla* spread spontaneously and no-P fertilizer and little labor were needed. Economic re-

turn from *Azolla* adoption, including cost savings in chemical fertilizers and weed control, was more than \$35/ha at 1981 prices. Conditions in the study area were exceptionally favorable, however, and should be viewed realistically. The authors concluded that the economic potential of *Azolla* is greatest where the opportunity cost of labor is low, and that labor cost becomes critical where wage rates approach \$2/d. Insect control was also an important economic limitation. If more than 200 g carbofuran ai/ha was needed to control insects, benefits were eliminated. In areas of the Philippines where conditions for *Azolla* growth were not favored by an exceptionally high level of available P, *Azolla* use was uneconomical (Rosegrant et al 1985, Rosegrant and Roumasset 1988).

Clearly, a case study in the Philippines is not enough to allow definite conclusions regarding *Azolla* economics, which may vary according to socioagricultural systems. Economic calculations should also consider the long-term benefits of *Azolla* as an organic fertilizer with the concomitant increase in soil OM and fertility, instead of only those costs directly comparable with commercial N fertilizer prices. The economics of integrated rice-fish-*Azolla* culture might be more favorable and should be considered.

### 7.3.5. Biotechnologies for the agronomic use of *Azolla*

*Azolla* strains exhibit a wide range of behavior with regard to environmental factors, P requirement, N<sub>2</sub> fixation, productivity, etc. The ability to combine favorable characters such as resistance to high temperature and insects, low P requirement, and erect growth (permitting higher productivity) would allow strains to be designed for specific agroecological conditions. For this purpose, recombination of different algal and plant symbionts and sexual hybridization between *Azolla* species proved feasible. *Anabaena* from *Azolla filiculoides* was recombined with *A. microphylla* and vice versa. Megasporecarps of each species were freed of their algal symbiont (Lin and Watanabe 1988), then an indusium of the other *Azolla* species, containing the corresponding *Anabaena*, was placed on the alga-free megasporecarps. Symbiosis was thus established with the newly forming sporophyte (Lin et al 1988).

The formation of *Azolla* hybrids requires that macrosporecarps and microsporecarps be obtained. Sporulation of many strains can be observed under natural conditions (Payawal and Paderon 1986), but no satisfactory method has yet been designed to induce sporulation at will. That is a major limiting factor for *Azolla* hybridization. Hybrids of *A. microphylla* (female parent) and *A. filiculoides* have been obtained (Wei et al 1986). The IRRI biofertilizer germplasm collection contains 23 hybrid strains obtained by algal transfer and 85 obtained by sexual hybridization (Watanabe et al 1992).

### 7.3.6. Conclusion

*Azolla* is a N<sub>2</sub>-fixing organic manure with N potential similar to that of legumes. It is easier to incorporate than other organic manure crops and grows

well with rice in flooded conditions. Environmental, technological, and economic factors limit its use. Problems in inoculum conservation, multiplication, and transport could be solved if *Azolla* could be propagated from spores. Temperature limitations and P requirements can be reduced by selecting cold- or heat-resistant strains with low P requirements and by split application of P fertilizer, whether limited to inoculum production or not. Economic limitations are important and need further evaluation. Economic calculations should also consider the long-term benefits of *Azolla* on soil fertility. *Azolla* has a potential not only as a green manure but as a multipurpose biofertilizer that can also be a weed suppressor, a K source through its ability to concentrate the element, an animal feed, and a primary producer in rice-fish-*Azolla* culture. That potential may further increase interest in *Azolla* use.

#### 7.4. Use of free-living cyanobacteria as biofertilizer

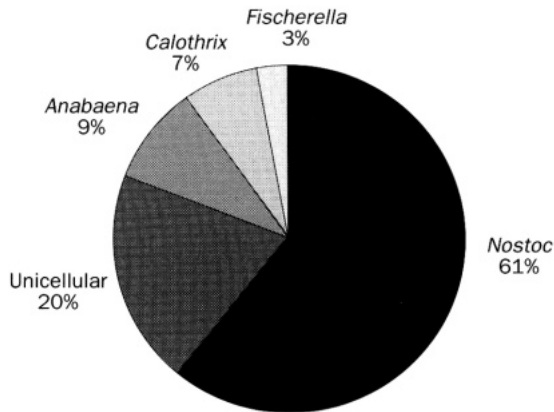
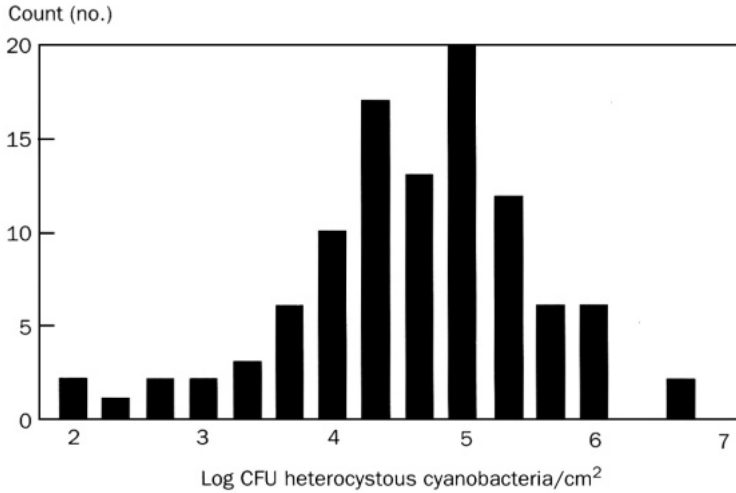
Cyanobacteria are photosynthetic, prokaryotic microorganisms, some of which are capable of N<sub>2</sub> fixation. Their main photosynthetic pigments are chlorophyll a, carotenes, and xanthophylls together with phycobiliproteins, c-phycocyanin (blue) and c-phycoerythrin (red). Due to the presence of these pigments and mucilage, the color of blue-green algae (BGA) in nature may range from dirty yellow, through various shades of blue green, to brown or black. Their range in vegetative form extends from simple unicells to multiseriate, true branching thalli.

Some cyanobacteria can fix N<sub>2</sub> because they contain nitrogenase, an O<sub>2</sub>-sensitive enzyme. This ability was first related to the presence of specialized non-O<sub>2</sub>-evolving cells called heterocysts in which the enzyme is protected from O<sub>2</sub>. But N<sub>2</sub>-fixing ability is also found in some unicellular and homocystous forms that fix N<sub>2</sub> under microaerophilic or anaerobic conditions.

The existence of an agronomic potential for cyanobacteria in rice cultivation was recognized by De (1939), who attributed the fertility of tropical ricefields to BNF by those organisms. Since then, long-term fertility experiments and N<sub>2</sub>-fixation measurements have confirmed the importance of cyanobacteria and other N<sub>2</sub>-fixing organisms in maintaining a moderate but constant rice production in fields receiving no N fertilizer. Many trials to increase yield by cyanobacteria inoculation have been conducted. Those are summarized in several reviews (Roger and Kulasooriya 1980, Roger and Reynaud 1982, Roger and Watanabe 1986, Roger et al 1993).

##### 7.4.1. Ecology of cyanobacteria in ricefields

7.4.1.1. *Occurrence of cyanobacteria in rice soils.* Early qualitative surveys reported a limited occurrence of N<sub>2</sub>-fixing cyanobacteria in rice soils (Watanabe 1959, Watanabe and Yamamoto 1971, Venkataraman 1975). Quantitative studies during the last decade, however, showed the consistent presence of N<sub>2</sub>-fixing cyanobacteria in soils under rice cultivation with an average value of  $1.5 \times 10^5$



7.1. Abundance and major taxa of N<sub>2</sub>-fixing cyanobacteria in 109 soils from 5 countries. CFU = colony-forming units (Roger et al 1987b).

colony forming units (CFU)/g dry soil, and a median of  $2.0 \times 10^4$  (Table 7.3). Enumerations in 102 samples of rice soils from India, Malaysia, the Philippines, and Portugal had a median of  $6.4 \times 10^5$  CFU/g dry soil. Dominant genera were those forming mucilaginous colonies: *Nostoc* and unicellular *Aphanothece* (Fig. 7.1). Cyanobacteria are probably ubiquitous in rice soils.

7.4.1.2. *Climatic factors.* As phototrophs, cyanobacteria are mainly restricted to the photic zone of the ricefield; however, viable propagules are found in the deeper layers of the soil. In dry tropics, very high light intensities, which are usually associated with high water temperature, can be detrimental to

**Table 7.3. Density of N<sub>2</sub>-fixing cyanobacteria in rice soils.**

Country	Samples		Colony-forming units/g soil dry weight				Reference	
	no.	% with cyanobacteria	Min	Max	Average	Median		
Thailand	100	... a	...	...	8.6	10 <sup>3</sup>	...	Araragi and Tangcham 1979b
Bangladesh	6	100	2 x 10 <sup>3</sup>	3 x 10 <sup>4</sup>	2 x 10 <sup>4</sup>	1 x 10 <sup>4</sup>	...	Bhuiyan et al 1981b
Senegal	15	100	8 x 10 <sup>1</sup>	2 x 10 <sup>6</sup>	5 x 10 <sup>5</sup>	8 x 10 <sup>3</sup>	...	Garcia et al 1973
Iraq	7	100	...	...	1 x 10 <sup>2</sup>	...	...	Hamdi et al 1978b
Philippines	61	100	3 x 10 <sup>2</sup>	3 x 10 <sup>6</sup>	3 x 10 <sup>5</sup>	2 x 10 <sup>5</sup>	...	IRRI 1985
SE Asia	25	100	1 x 10 <sup>3</sup>	1 x 10 <sup>7</sup>	1 x 10 <sup>6</sup>	1 x 10 <sup>5</sup>	...	Kobayashi et al 1967b
Thailand	40	100	1 x 10 <sup>1</sup>	1 x 10 <sup>5</sup>	...	8 x 10 <sup>3</sup>	...	Matsuguchi et al 1975b
India	16	100	6 x 10 <sup>3</sup>	4 x 10 <sup>6</sup>	9 x 10 <sup>5</sup>	5 x 10 <sup>5</sup>	...	Roger et al 1985a
Philippines	14	100	8 x 10 <sup>3</sup>	1 x 10 <sup>6</sup>	2 x 10 <sup>5</sup>	5 x 10 <sup>4</sup>	...	Roger et al 1986a
Asia	102	100	1 x 10 <sup>2</sup>	8 x 10 <sup>6</sup>	3 x 10 <sup>5</sup>	6 x 10 <sup>4</sup>	...	Roger et al 1987b
India	10	100	2 x 10 <sup>3</sup>	2 x 10 <sup>5</sup>	8 x 10 <sup>4</sup>	7 x 10 <sup>4</sup>	...	Saha and Mandal 1979b
Cambodia	...	...	1 x 10 <sup>5</sup>	1 x 10 <sup>6</sup>	...	...	...	Suzuki and Kaway 1971b
Pooled data	396	...	1 x 10 <sup>1</sup>	1 x 10 <sup>7</sup>	2 x 10 <sup>5</sup>	2 x 10 <sup>4</sup>	...	

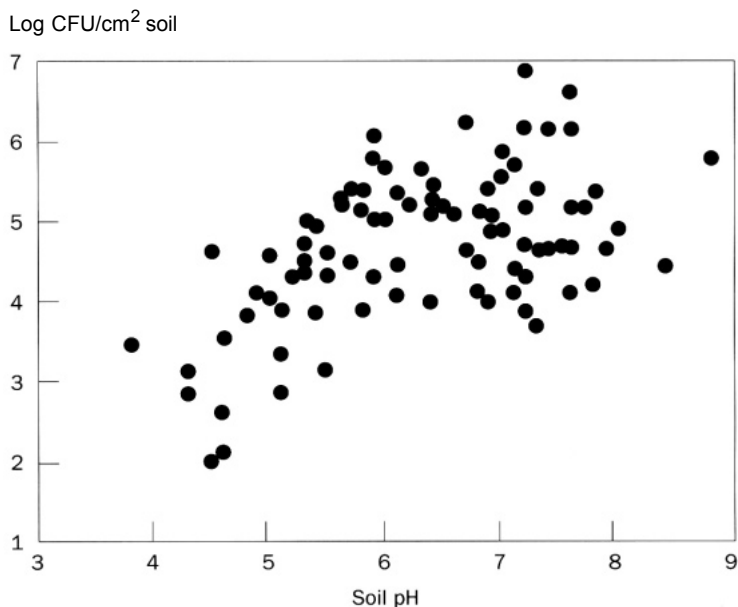
<sup>a</sup>... = not available. <sup>b</sup> Cited by Roger et al (1987b).

cyanobacteria. In humid tropics, where light intensity is not as high, better growth is usually observed during DS when solar radiation is high, than during WS when solar radiation is lower and there is more disturbance by rain. High temperatures favor cyanobacteria, but temperatures higher than 40 °C are detrimental (Ichimura 1954, Mori 1963, Subrahmanyam et al 1965). The high capacity of cyanobacteria to withstand long periods of desiccation may explain why their relative abundance increases with the duration of dry fallow. The survey of 102 rice soils showed a higher incidence of spore-forming *Nostoc* in dry soils (80%) than in wet soils (47%) (Roger et al 1987b).

Rain is usually accompanied by a decrease in light intensity and ambient temperature, an increase in water turbidity, and rapid changes in water pH, all of which limit cyanobacteria growth and photodependent N<sub>2</sub> fixation.

7.4.1.3. *Soil factors.* Soil properties that have been most frequently associated with cyanobacteria occurrence and blooming are pH and available P content.

Positive correlations between pH and occurrence, abundance, or growth of cyanobacteria were reported by several authors (Garcia et al 1974, Okuda and Yamaguchi 1955, Okuda and Yamaguchi 1956, Roger and Reynaud 1977). An extensive survey (Roger et al 1987b) showed that the correlation was significant only in soils having a pH lower than 6.5 (Fig. 7.2). Roger and Kulasooriya (1980) cite 12 experiments in which the positive effect of high pH on cyanobacteria growth is further demonstrated by an increased growth of



7.2. Correlation between soil pH and abundance of N<sub>2</sub>-fixing cyanobacteria (Roger et al 1987b).

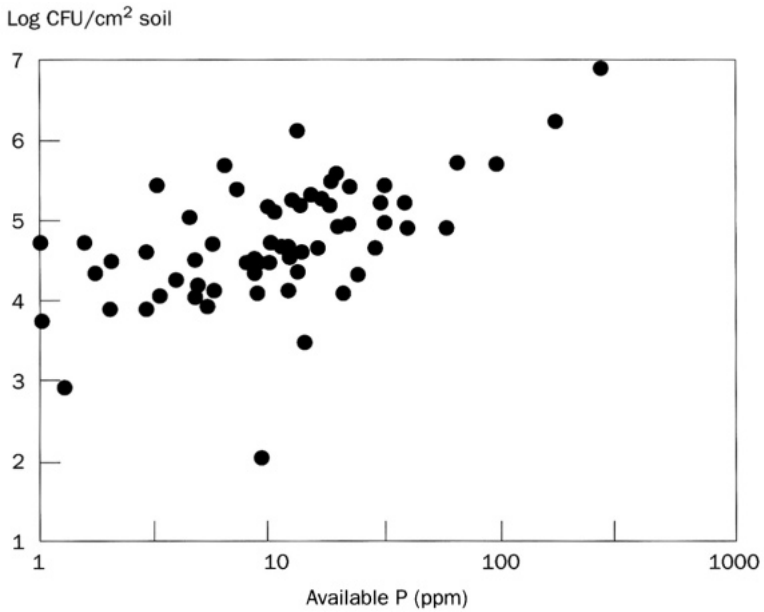
cyanobacteria and  $N_2$  fixation after liming. There are, however, reports on the presence of cyanobacteria strains in acidic soils (Bunt 1961, Prasad et al 1978, Roger et al 1986c, Roger et al 1987b). *Aulosira fertilissima* and *Calothrix brevissima* were reported to be ubiquitous in Kerala (India) ricefields where pH ranged from 3.5 to 6.5 (Aiyer 1965). The development of a dense algal bloom on an acidic soil (pH 5.5) was observed in Japan after surface application of straw (Matsuguchi and Yoo 1981).

Next to pH, the most decisive soil factor is available P (Roger and Kulasoorya 1980), which is positively correlated with cyanobacteria abundance (Matsuguchi and Tangcham 1974, Roger et al 1987b) (Fig. 7.3). The analysis of laboratory and field samples of cyanobacteria (Fig. 7.4) shows a highly significant positive correlation between N and P contents expressed on ash-free basis. The general shape of the curve shows that at P contents higher than 1% there was no more increase in N content, indicating a *luxury consumption* in P. The optimal value of 1% was attained in laboratory cultures only. Cyanobacteria grown on soil and natural samples had concentrations lower than 0.5%, confirming that P availability is a major limiting factor for cyanobacteria growth in natural environments (Roger et al 1986b).

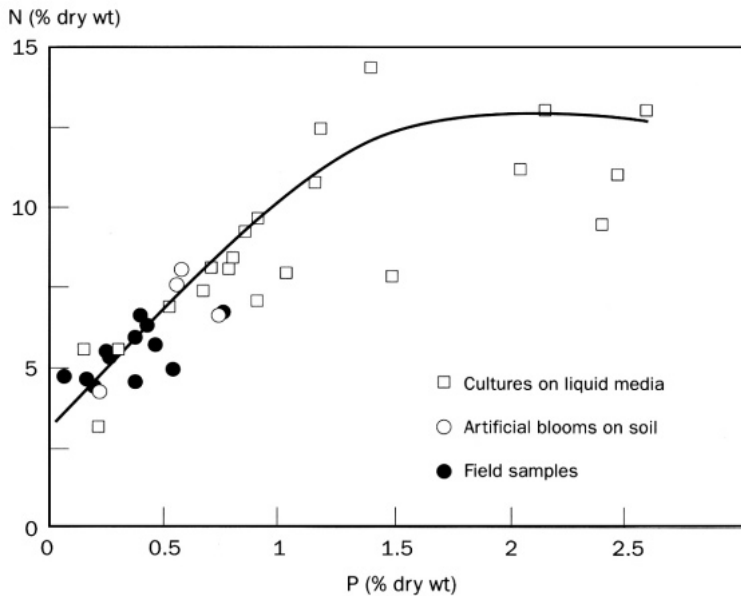
Little is known about the effect of other soil properties. A laboratory study of 12 rice soils (Wilson and Alexander 1979) showed that cyanobacteria growth and  $N_2$ -fixing activity were correlated with the levels of extractable K, Ca, and Mg, but not with extractable phosphate and Fe. Cyanobacteria growth or abundance does not seem to be correlated with soil OM content or texture (Okuda and Yamaguchi 1955, 1956; Roger et al 1987b).

7.4.1.4. *Biotic factors.* Viral and microbial pathogens of cyanobacteria and antagonism between cyanobacteria and other PAB components have been observed; however, the major biotic limiting factor for cyanobacteria is grazing (see sect. 5.3.). Grazing by invertebrates limits cyanobacteria growth, may cause blooms to disappear (Venkataraman 1961), and may prevent the establishment of cyanobacteria inocula (Hirano et al 1955, Watanabe et al 1955). Because cyanobacteria forming mucilaginous colonies (*Aphanothece*, *Nostoc*, *Gloetrichia*) are less susceptible to grazing than strains that do not (Grant et al 1985), grazing leads to the dominance of mucilaginous colonial cyanobacteria in wetland ricefields (Fig. 7.1).

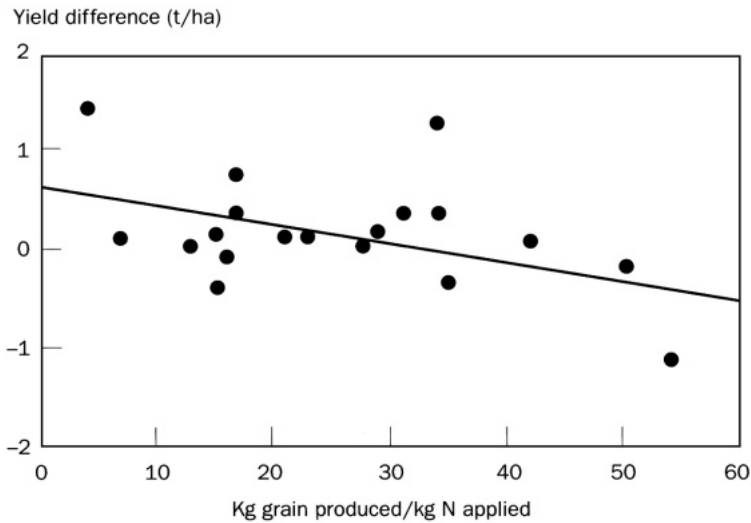
7.4.1.5. *Effects of N fertilizer on  $N_2$  fixation by cyanobacteria.* Roger and Kulasoorya (1980) list seven references reporting that N fertilizer inhibited cyanobacteria growth in ricefields, but that also suggest inhibition in situ might not be as marked as that in vitro. Studies at IRRI (IRRI 1987b, 1988; Roger et al 1988) showed 1) a strong inhibition of photodependent ARA by broadcast urea in about 75% of 60 cases, while in others a significant ARA was recorded, and 2) a negative correlation between ARA and rice yield, which indicates that photodependent  $N_2$  fixation in plots where N was broadcast was significant only when N efficiency (kg rice produced/kg N applied) was low. Similarly, trials at 22 sites in India (Pillai 1980) showed a negative correlation be-



**7.3.** Correlation between soil available P and abundance of N<sub>2</sub>-fixing cyanobacteria (Roger et al 1987b).



**7.4.** Correlation between P and N contents (ash-free basis) in sample of cyanobacteria from various origins (Roger et al 1986b).



7.5. Correlation between yield change in experimental fields inoculated with cyanobacteria and fertilizer efficiency (25 kg N/ha) at 22 sites (calculated and drawn from data of Pillai 1980).

tween N efficiency and the effect of cyanobacteria inoculation in fertilized plots (Fig. 7.5). Cyanobacteria inhibition by N fertilizer might be more indirect than direct. That aspect is developed in sect. 6.2.3.

#### 7.4.2. Estimation of the nitrogen potential of cyanobacteria in ricefields

The potential of cyanobacteria as a N source is indirectly estimated by measuring 1) the cyanobacteria biomass and its N content, 2)  $N_2$ -fixing activity, and 3) increase in grain yield in inoculation experiments with cyanobacteria.

7.4.2.1. *Evaluation of cyanobacteria biomass and nitrogen content.* Extensive data on phytoplankton and cyanobacteria biomass and their composition are presented in sect. 4.2.1 and sect. 4.3.1. Because of the wide range of DM (0.2-14%) and ash (31-71%) concentrations in field-grown cyanobacteria, N in 1 t of fresh cyanobacteria averages 1.25 kg, but may vary from 0.1 kg to 4 kg (Roger et al 1986b). Reported  $N_2$ -fixing cyanobacteria biomasses range from a few kg to 0.5 t dw/ha (Roger et al 1987a). Assuming a maximum biomass of 0.5 t dw/ha and using average ash and N contents obtained for field samples, the potential average contribution of a cyanobacteria bloom is about 15 kg N/ha.

A calculation using average values for DM, ash, and N contents in cyanobacteria shows that the average biomass corresponding to 10 kg N is about 8 t fw. That is equivalent to a continuous layer of about 1 mm of algal material over 1 ha of ricefield. In other words, an algal bloom of agronomic significance is visible to the naked eye.

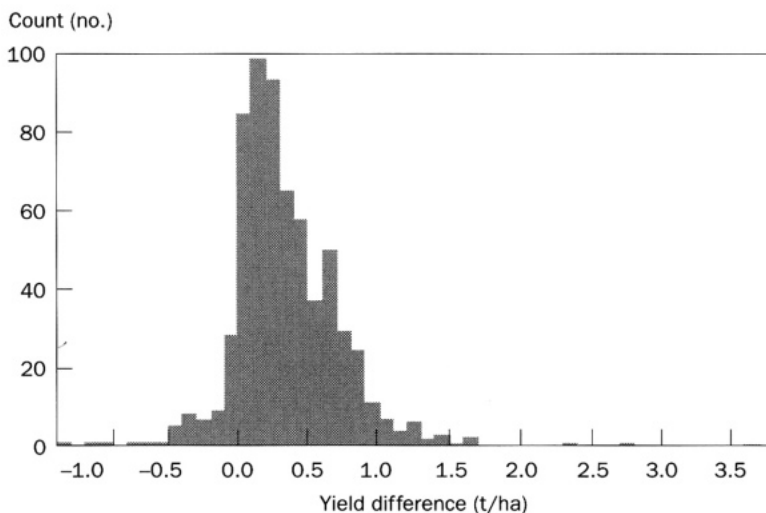
Available data indicate that a visible growth of cyanobacteria usually corresponds to less than 10 kg N/ha and a very dense bloom may correspond to 10-20 kg N/ha (Fig. 4.1). Nitrogen contribution by cyanobacteria is the result of nutrient turnover of the standing biomass, for which no data are yet available. However, the observation that cyanobacteria usually do not bloom more than twice during a crop cycle indicates a rough maximum potential of 30 kg N/ha per crop.

Assuming that all C input in the floodwater and surface soil is through N<sub>2</sub>-fixing cyanobacteria (an obvious overestimation) and using 1) an input of 0.6 t C/ha per crop (Saito and Watanabe 1978), and 2) an average C-N ratio of 8 (Roger et al 1986b), the theoretical maximum contribution of N<sub>2</sub>-fixing cyanobacteria could be 75 kg N/ha per crop.

7.4.2.2. *Measurements of N<sub>2</sub>-fixing activity.* Estimates are mostly extrapolated from ARA measurements (see sect. 4.6.1). Values published before 1980 range from a few to 80 kg N/ha per crop and average 27 kg N/ha per crop (Roger and Kulasooriya 1980). Three groups of 60 estimates each in experimental plots at IRRI averaged 20 kg N/ha per crop in no added N control plots, 8 kg in plots with broadcast urea, and 12 kg in plots where N was deep-placed (Roger et al 1988).

7.4.2.3. *Field experiments on cyanobacteria inoculation.* Inoculation experiments provide indirect information on the overall potential of cyanobacteria. The effects on rice yield of soil inoculation by cyanobacteria were first reported by Watanabe et al (1951): a 25% increase in a poorly drained ricefield inoculated with *Tolypothrix tenuis*. Several authors reported increases of more than 200% from pot trials (Singh 1961, Sundara Rao et al 1963). Subsequent studies have indicated much lower increases in the field than in pot trials, even where comparative studies have been made (C. Y. Huang 1978). In experiments published before 1980, the relative increase in yield over the control averaged 28% in pot experiments and 14% in the field, when algal inoculation was effective (Roger and Kulasooriya 1980). Possible causes for overestimated effects of cyanobacteria inoculation in pot experiments include the strong reduction of grazer populations (dry soil, depleted of fauna, is usually used), less climatic disturbance, and a mechanical effect at the pot wall, where cyanobacteria may grow profusely.

Figure 7.6 and Table 7.4 present the analysis of a bibliographic compilation of field data (634 experiments) reporting yield values in both a noninoculated control and the corresponding inoculated treatment at the same fertilizer level (Roger 1991). Results show large variability in yield between inoculated and noninoculated plots, with some surprising extreme values and a CV>100%. Average difference is +337 kg grain/ha or +11.3%. However, a very dissymmetrical histogram (Fig. 7.6) and a standard deviation close to the mean (Table 7.4) indicate that the median (+257 kg grain/ha or +7.9%) is a better index of the average effect of inoculation than the mean.



7.6. Histogram of the difference in yield between plots inoculated with cyanobacteria and noninoculated plots, calculated from 634 data reported by various authors (Roger 1991).

**Table 7.4. Statistics of absolute and relative yield differences between plots inoculated with cyanobacteria and controls (Roger 1991).<sup>a</sup>**

	Difference between control and inoculated plots	
	Absolute (kg/ha)	Relative (%)
Number of observations	634	634
Maximum	3700	168.2
Minimum	-1280	-19.3
Average	337	11.3
Median	257	7.9
Standard deviation	398	16.0
CV (%)	118	141

<sup>a</sup> Data compiled from 41 references.

The Student's-Fischer t-test for paired samples ( $df = 663$ ,  $t = -3.03$ ) shows a statistically significant difference in average yield between inoculated (3.9 t/ha) and noninoculated (3.6 t/ha) plots at  $p < 0.01$ . Only 17% of the experiments, however, report a statistically significant difference between inoculated plots and controls. That indicates that 1) the response to algal inoculation varies, 2) the response is small, and 3) the experimental error is larger

than the response. The most common design for BGA inoculation experiments has been 4- × 4-m plots with four replicates, which usually gives a CV>10%, and a minimum detectable difference of 14.5% (Gomez 1972), which is larger than the average yield increase reported after algal inoculation (average 11.3%, median 7.9%). It is probable that in many cases unsuccessful trials were not reported. When they were mentioned, it was without data that could explain the possible reason for failure. For example, a report of a multilocation trial (Pillai 1980) indicates that the 22 sets of data presented notwithstanding, "the results from many other locations . . . were not received because of the failure of multiplying cyanobacteria at these locations."

Biomass estimates, ARA measurements, and inoculation experiments indicate that as an additional source of N for rice, cyanobacteria have a maximum potential of about 30 kg N/ha, which may translate to a yield increase of 300-450 kg/ha.

#### **7.4.3. Effects of cyanobacteria on rice**

Cyanobacteria may increase yields by providing the crop with N, possibly by producing growth-promoting substances and improving P availability and soil properties. However, in most experiments, only grain yield was measured. No data regarding environmental conditions, cyanobacteria establishment, algal biomass, or N<sub>2</sub>-fixing activity in successful inoculation experiments are available. Therefore, reasons for the yield increase are still unclear, especially in cases when a beneficial effect was observed with high levels of N fertilizer, which reportedly inhibit cyanobacteria growth (Roger et al 1980).

7.4.3.1. *Use of cyanobacteria nitrogen by the rice plant.* Nitrogen fixed by cyanobacteria is released through exudation or decomposition after the cells die. No information is available on N exudation by cyanobacteria under field conditions. Considering, however, that a cyanobacteria bloom of agronomic significance markedly increases floodwater pH (Roger et al 1987a), it is foreseeable that during the buildup of a bloom, most of the excreted N will be either reimmobilized by the microflora or lost by NH<sub>3</sub> volatilization. Release through microbial decomposition after the death of the algae appears to be the principal means by which N is made available to the crop (Roger and Kulasooriya 1980). Death of cyanobacteria is associated with 1) grazing, 2) incorporation into soil during weeding, 3) biomass decline resulting from competition, and 4) desiccation at the end of the crop cycle. Microcrustaceans and gastropods are directly responsible for the breakdown of the cyanobacteria biomass. Protozoans and rotifers also recycle nutrients from decaying cyanobacteria. Tubificid worms expedite translocation to deeper soil layers (Grant and Seegers 1985a,b).

Susceptibility to decomposition varies with cyanobacteria composition. The C:N is negatively correlated with the percentage of N mineralized, which may range from 30 to 65% in 21 d (Roger et al 1986b). When looking at cyanobacteria as a source of N for rice, the C-N ratio is a major factor deter-

mining N mineralization. Values from blooms produced on soil and natural samples range from 5.3 to 11.6 and average 8 (Table 4.6). That indicates that cyanobacteria have a better N availability than organic fertilizers such as FYM and green manures.

Because a significant part of the cyanobacteria biomass dries up at the end of the crop cycle, cyanobacteria growth sometimes has a residual effect on, rather than an immediate benefit to, the standing rice crop. Late decomposition of the cyanobacteria biomass and the resulting late availability of N to rice might increase N content of the grain without increasing yield (Grant et al 1985).

Recovery of cyanobacteria N by rice varies from 13 to 50%, depending on the nature of the material, the method of application, and the presence or absence of soil fauna (Table 4.10). Use of N from cyanobacteria by rice is more efficient when fresh cyanobacteria biomass is incorporated into the soil (see sect. 4.7.6).

Incorporated cyanobacteria N has a residual effect, 4-7% being recovered in the next crop (Tirol et al 1982, Grant and Seegers 1985a). Pot experiment with two successive crops (Tirol et al 1982) showed that for the first crop algal  $^{15}\text{N}$  was less available than  $(\text{NH}_4)_2\text{SO}_4$ , but over two crops its availability was similar. After two crops, 57% of  $^{15}\text{N}$  from cyanobacteria and 30-40% of  $^{15}\text{N}$  from  $(\text{NH}_4)_2\text{SO}_4$  remained in the soil, suggesting that algal N is less susceptible to losses than mineral N. That indicates the slow-release nature of algal N; however, the low C-N ratio (5-8) of cyanobacteria gives it better N availability than that of other organic fertilizers.

7.4.3.2. *Plant growth regulators.* Besides increasing N fertility, cyanobacteria have been said to benefit rice plants by producing plant growth regulators (PGR). Roger and Kulasooriya (1980) cite 11 references where the additive effects of cyanobacteria inoculation in the presence of N fertilizers were interpreted as an effect of a PGR produced by the algae. Such interpretations are obviously hypothetical. More direct evidence of PGR effects has come primarily from treatment of rice seedlings with algal cultures or their extracts. Roger and Kulasooriya (1980) cite 12 references reporting that soaking of seeds or seedlings in cyanobacteria cultures or extracts resulted in some combination of the following effects: enhanced germination; faster growth; early seedling recovery; increased root growth; prolonged tillering; stimulated vegetative growth; and increase in length and number of ears, in number of grains per ear, and in weight and protein content of the grains. However, when 133 unialgal strains isolated from sites in Africa (not all ricefields) were tested for their effects on rice germination and growth, 70% had a negative effect on germination and only 21% a stimulatory effect; many *Nostoc* strains had a negative effect (Pedurand and Reynaud 1987). The PGR-like effects of cyanobacteria cultures were likened to those of vitamin B12, gibberellins, and amino acids, but, despite the numerous reports of algal PGR effects, none shows the isolation and characterization of a microalgal PGR (Metting and

Pyne 1986). Beneficial effects of algal inoculation might be due partially to PGR, but the relative importance of their contribution to rice productivity is still unknown.

7.4.3.3. *Other effects on rice.* Cyanobacteria colonization of ricefields prevented the growth of weeds (Subrahmanyam et al 1965). Excretion of organic acids by cyanobacteria increased P availability to rice (Arora 1969). Field inoculation (Aiyer et al 1971) and soaking of the rice seeds (Jacq and Roger 1977) decreased sulfide injury to the rice crop. That was explained by an increased O<sub>2</sub> content of the medium that made it unfavorable to sulfate-reducing bacteria and by a faster growth of rice that increased its resistance to sulfide.

#### 7.4.4. Use of cyanobacteria in rice cultivation

Until recently, studies on agronomic use of cyanobacteria have emphasized algal inoculation with foreign strains selected in the laboratory. That arose from the earlier belief that N<sub>2</sub>-fixing cyanobacteria were not normally present in many ricefields. Recent results show that N<sub>2</sub>-fixing cyanobacteria are probably ubiquitous in rice soils; therefore, research should pay more attention to agricultural practices that enhance the growth of indigenous cyanobacteria.

7.4.4.1. *Algal inoculation.* Experimental cyanobacteria inoculation of ricefields initiated in Japan by Watanabe et al (1951) was subsequently abandoned there. Most applied research on cyanobacteria inoculation has been conducted in India where the All-India Coordinated Project on Algae was initiated in 1977. To a lesser extent, research is also conducted in China, Egypt, and Myanmar. A similar technique of growing cyanobacteria inocula in shallow, open-air ponds is used in Egypt, India, and Myanmar (Venkataraman 1981). This simple and cheap method uses a multistrain starter inoculum produced from laboratory cultures and propagated, on the spot, in shallow trays with 5-15 cm water, about 4 kg soil/m<sup>2</sup>, 100 g superphosphate/m<sup>2</sup>, and insecticide. If necessary, CaCO<sub>3</sub> is added to adjust soil pH to 7.0-7.5. In 1-3 wk, an algal mat develops, which is then allowed to dry. Algal flakes are scraped off and stored in bags for use in fields at a rate of 10 kg/ha (Venkataraman 1981).

Currently, cyanobacteria inoculation is used on a trial-and-error basis. Its use is limited by a lack of basic knowledge regarding the factors involved in the establishment of N<sub>2</sub>-fixing blooms and the success or failure of inoculation. No method to estimate the chance of success of inoculation in a given agroecosystem is available. The factors underlying yield increases reported after successful algal inoculation are still not clearly understood and their effects are not quantified. In most experiments only grain yield was measured. No published study describing a significant increase in yield after algal inoculation includes N<sub>2</sub>-fixation measurements or even cyanobacteria biomass measurements. That is surprising, considering that a bloom of agronomic significance is visible to the naked eye and can be easily sampled.

Little attention has been paid to inoculum quality and establishment. Published methods of inoculum production do not include tests of composi-

tion and viability. Available data show that the density of CFUs of N<sub>2</sub>-fixing strains in soil-based inocula may vary from 10<sup>3</sup> to 10<sup>7</sup>/g dw and constitute only an average 13% of total algae (Roger et al 1987b). Very few published inoculation experiments examined the establishment of the inoculum. The inoculum propagation method proposed for farmers has a high chance of producing an inoculum of indigenous strains rather than of strains of the starter.

Reports on the adoption of cyanobacteria inoculation are somewhat controversial, but even with the most optimistic evaluations, adoption seems to be restricted to a limited area in a few Indian states, and possibly in Myanmar. Reviewing the adoption of biofertilizers in India in 1980, Pillai (1980) wrote, "Apart from the work carried out at research stations very little organized work on development of the material for being adopted by the farmers has been taken up, especially in areas where it could be of potential benefit." Venkataraman (1982) wrote, "A conservative estimate suggests that about two million hectares under rice are currently covered with algal biofertilizer technology." The same year, Subba Rao (1982) reported that the production capacity of cyanobacteria flakes in India was around 40 t/yr, which was approximately 0.01% of the total inoculum requirement for the country. A report of the Agricultural Economics Research Center of the University of Madras (University of Madras 1982) indicated that despite an official radio and print publicity campaign, cyanobacteria use remained at the trial level; in many trials the algae inoculum did not multiply. In 1986, about 4,200 ha of Egyptian ricefields were inoculated with cyanobacteria (Alaa el-Din and Shaalan 1989). In 1985, Roger et al reported that cyanobacteria inoculation was adopted in only two states of India (Tamil Nadu and Uttar Pradesh) where inoculated fields were only a few percent of the total area under rice (Roger et al 1985a). Cyanobacteria inoculation technology is still more at the experimental level of large-scale field testing rather than at a popularization stage. The limited adoption of algal inoculation by farmers probably reflects the low and erratic increases in yield obtained, which can be expected from a technology used on a trial-and-error basis.

A study of the economics of cyanobacteria use by 40 farmers in Tamil Nadu (University of Madras 1982) found no significant difference in the average cost of cultivation between crops using (\$247/ha) and not using (\$246/ha) cyanobacteria, but the average return to cyanobacteria use was only \$4/ha. Only 5 of 40 farmers reported that cyanobacteria had increased yield. Among 10 farmers not using cyanobacteria, 4 were unaware of the technology, 2 were aware of it but could not get any inoculum, and 4 said they were not convinced about the yield-increasing quality of the algae.

7.4.4.2. *Soil reclamation by cyanobacteria.* A method using natural blooms of cyanobacteria for reclaiming saline and alkaline unproductive *Usar* soils, found extensively in India, was described by Singh (1950). These soils are

usually very deep; their reclamation by the addition of gypsum is possible but rather expensive.

The method arose from the observation that cyanobacteria form a thick mat on *Usar* soils of northern India during WS, while other plants fail to grow. Laboratory experiments showed marked improvements in the soil characteristics after algal growth when compared with a control covered with black cloth : pH decreased from 9.2 to 7.5, and large increases in OM (69%), total N (46%), water-holding capacity (35%), exchangeable Ca (31%), and soluble P (about 16 times as much) were observed. Other experiments confirm a beneficial effect of cyanobacteria growth on saline soils (Kaushik et al 1981). The reclamation process consisted of dividing the land into plots of less than 0.4 ha and enclosing them with an earth embankment before the rains started. The resulting water conservation permitted the establishment of a thick mat of N<sub>2</sub>-fixing cyanobacteria whose incorporation increased soil fertility and structure and improved drainage. After a year, a transplanted rice crop yielded 1.6-2.2 t/ha, whereas normally rice did not grow on such soils. Reclamation allowed the growth of sugarcane after 3 yr. Subsequent improvements of the technique were proposed (Singh 1961) including repeating the process where irrigation water is available, contour farming to increase water penetration, and inoculation of the soil with cyanobacteria. But adoption of this technique has not spread.

7.4.4.3. *Reconsidering agronomic use of cyanobacteria.* Methods for using cyanobacteria in rice cultivation need to be reconsidered in view of the results of the agroecological studies of the last decade regarding 1) the occurrence of cyanobacteria in rice soils, 2) the composition of soil-based inocula of cyanobacteria, and 3) the establishment of cyanobacteria inoculum in soils.

Because of the belief that N<sub>2</sub>-fixing cyanobacteria were not common in many rice soils, research on methods for using cyanobacteria in rice cultivation has focused on inoculation. However, surveys have shown that heterocystous cyanobacteria are ubiquitous in rice soils at densities averaging  $5 \times 10^4/\text{cm}^2$  (Roger et al 1987b).

The study of the ratio of indigenous heterocystous cyanobacteria in 102 soils (1st cm of soil over 1 ha) to heterocystous cyanobacteria contained in the recommended dose of 22 soil-based inocula (10 kg/ha) showed that in 90% of the cases, indigenous cyanobacteria were more abundant than cyanobacteria in the inoculum. In 59% of the cases, the ratio of indigenous cyanobacteria to inoculated cyanobacteria was higher than 100 (Roger et al 1987b).

Results also show the infrequent establishment of nonindigenous strains inoculated into various soils, even when grazers were controlled (Grant et al 1985, Reddy et al 1986, Reynaud and Metting 1988). Although cyanobacteria inoculum in five Philippine wetland soils persisted for at least 1 mo, their growth as a bloom was rare (1 out of 10 cases). Blooms developed on all soils when grazers were controlled, but were mostly of indigenous strains (Reddy and Roger 1988).

This suggests that some attention should be paid to practices that enhance the growth of indigenous strains already adapted to the environment.

The apparent ubiquity of heterocystous cyanobacteria in rice soils, however, does mean that inoculation is unnecessary. Inoculation with an inoculum of indigenous strains might be useful because the accumulation of P by the propagules of the cyanobacteria inoculum (produced with high levels of P) gives them an initial advantage over the indigenous propagules, which are usually P-deficient (Roger et al 1986b). Since spore germination is photodependent (Reddy 1983), inoculated propagules applied on the soil surface might germinate better than the indigenous ones mixed with the soil.

7.4.4.4. *Agricultural practices other than inoculation to enhance cyanobacteria growth.* The growth of N<sub>2</sub>-fixing cyanobacteria in ricefields is most commonly limited by low pH, P deficiency, grazing, and broadcasting of N fertilizer. Cultural practices that alleviate these limiting factors often favor cyanobacteria growth.

Liming is known to favor cyanobacteria growth (Roger and Kulasooriya 1980, Watanabe and Cholitkul 1982) but is rarely economical.

Many laboratory experiments have demonstrated that P application stimulates photodependent N<sub>2</sub> fixation and cyanobacteria growth (Roger and Kulasooriya 1980), especially in acid soils (Cholitkul et al 1980, Wilson and Alexander 1979). However, reported efficiency (2.3 g N/g P) (Cholitkul et al 1980) is low. Split application was more efficient than basal application (IRRI 1986).

Several experiments show that the control of grazer populations, especially ostracods, with synthetic or botanical pesticides (Grant et al 1983b,c, 1985) stimulates cyanobacteria growth and photodependent N<sub>2</sub> fixation. A pot experiment showed a highly significant positive N balance in soils treated with neem *Azadirachta indica* seeds, which have insecticidal properties. When algal growth was prevented with a black cloth covering the soil, only a small N balance was maintained. Neem had no direct effect on the plant; its action was attributed to the alleviation of ostracod grazing pressure on cyanobacteria (Grant et al 1984). In microplot experiments, N accumulation at the soil surface layer increased 1-3.5 times when grazers were controlled with insecticide, the rate depending on the soil type and algae growing on it (Roger et al 1987a). In a field experiment, control of grazing by neem seeds and cultural practices increased cyanobacteria biomass threefold and ARA tenfold. Significant increases in rice grain protein (37%) occurred when heterocystous algae bloomed early in the rice cultivation cycle and grazing control was maintained over 40 d (Grant et al 1985).

The ideal control of grazers could be a manipulation of the floodwater ecosystem to delay grazer growth to 1) arrest early consumption of N<sub>2</sub>-fixing primary producers, 2) permit the development of an efficient N<sub>2</sub>-fixing cyanobacteria bloom at the beginning of the crop cycle, and 3) allow grazer

growth thereafter and early algal N recycling to ensure its best use by the plant.

Grazers can be controlled with conventional pesticides, but their cost is prohibitive for the result achieved. Botanical pesticides might be more economical. Crushed nuts of the neem tree readily controlled ostracods for 20 d (Grant et al 1983c). Nuts, berries, or shells of *Phytolacca dodecandra*, *Croton tiglium*, and *Anacardium occidentale* controlled some snail populations (McCullough et al 1980, Webbe and Lambert 1983). A major problem with botanical pesticides is the variability of their insecticidal properties, which depend upon the growth conditions and the time and methods of harvest and conservation. Inconsistent effects of neem seed applications were obtained in the experiments conducted at IRRI (Roger 1991).

Wetting and drying fields at selected times is an alternative way of arresting the reproduction of microcrustaceans and molluscs in wetland soils.

In eutrophic lakes, introduction of dense populations of planktivorous and benthivorous fish resulted in low numbers of benthic fauna and planktonic cladocerans and in the development of cyanobacteria blooms. Introduction of selected fish species in rice-fish cultivation might increase the N fertility of the ecosystem by favoring photodependent  $N_2$  fixation (Andersson et al 1978).

The study of different methods of N fertilizer application on the algal flora and photodependent  $N_2$  fixation has shown that surface broadcast application of N fertilizer, which is widely practiced by farmers, not only inhibits photodependent  $N_2$  fixation but favors the growth of green algae, which increases floodwater pH and causes fertilizer N losses by  $NH_3$  volatilization (Fillery et al 1986). In contrast, the deep placement of N fertilizer decreases its inhibitory effect on cyanobacteria and reduces N losses by volatilization (Roger et al 1980, Fillery et al 1986). Experiments at IRRI (Table 6.3) show a smaller inhibition of ARA in plots where urea was deep placed than in plots where it was surface applied. The inhibition observed with deep-placed N was variable and was probably related to N contamination of the floodwater during placement. When N was placed without standing water, average ARA was similar to that in the no-N controls, although inhibition was still marked when N was placed with standing water in the field. Another possible method would be to delay N fertilizer application to allow the growth of a  $N_2$ -fixing algal bloom at the early stages of the crop, but the resulting effects—especially on N losses from fertilizer applied in an algal-rich water—have to be tested.

While the effectiveness of those practices in increasing cyanobacteria growth and ARA has been established, no experiment has yet quantified the relative contribution of the increased cyanobacteria activity and the direct effect of the practice to yield increase, when observed.

#### 7.4.5. Biotechnologies for the agronomic use of free-living cyanobacteria

Since emphasis has been placed on the prokaryotic nature of cyanobacteria, significant progress has been made in their genetics, including, from an applied point of view, the transformation of *Anacystis* for herbicide resistance studies (Golden and Haselkorn 1985). One can speculate on the possibility of selecting or designing more efficient strains for field inoculation. Laboratory screening for N<sub>2</sub>-fixing activity might not be very useful per se, because there is little reason why such activity should correlate well with establishment in situ. Most fast-growing strains, with doubling times of 5-12 h, belong to *Anabaena*, have short filaments, and do not form mucilaginous colonies (Antarikanonda and Lorenzen 1982, Huang TanChi 1983). Such strains will certainly be susceptible to grazing. A study of 12 strains showed that those with high N<sub>2</sub>-fixing activity in vitro were not the best for inoculation because they could not establish themselves in the soil (Huang TanChi 1983).

A nitrogenase-depressed mutant of *Anabaena variabilis*, which excretes NH<sub>4</sub><sup>+</sup> into the medium, was found to be more efficient in providing N to rice in a N-free gnotobiotic culture than the parent strain (Latorre et al 1986). Studies at IRRRI (1989), however, showed that the strain is not competitive and did not multiply when inoculated in soil microplots.

Biological engineering of cyanobacteria is currently limited to unicellular strains, which are morphologically, physiologically, and ecologically different from the N<sub>2</sub>-fixing strains considered for inoculating ricefields. Probably, *super* N<sub>2</sub>-fixing cyanobacteria can be selected or designed and grown in test tubes, but the characteristics that will enable them to survive, develop, and fix N<sub>2</sub> as programmed in ricefields are still largely unknown. The immediate need is for a better understanding of cyanobacteria ecology but over the long term, genetic engineering may also contribute to the agronomic use of cyanobacteria.

#### 7.4.6. Conclusion

Numerous field inoculation experiments provide indirect information on the overall potential of cyanobacteria. When inoculation is effective, yield increases average 200-300 kg/ha per crop, equivalent to the application of 20-30 kg N/ha. Inoculation is less efficient in the presence of broadcast N fertilizer, but still may increase rice yield.

In terms of N<sub>2</sub> fixed, cyanobacteria have less potential than green manure (Roger and Watanabe 1986), but cyanobacteria inoculation, when successful, is a very low-cost technology with a cost-to-benefit ratio far more favorable than that of green manure (Venkataraman 1981).

Because of a very limited knowledge of factors that allow cyanobacteria to bloom in ricefields, cyanobacteria inoculation is conducted on a trial-and-error basis, and its effects seem to be erratic and frequently small. That may explain why algal inoculation, known for at least 30 yr, is practiced on only a limited hectareage in a few Indian states and possibly in Myanmar. Recent

studies show that N<sub>2</sub>-fixing cyanobacteria are present in ricefields at a much higher rate than was previously thought. Identifying and alleviating limiting factors in fields where cyanobacteria are present but do not bloom might be enough to make use of their potential. That is also a requisite for establishing strains of inoculum, if inoculation is needed.

Cyanobacteria have a potential in low-input farming systems where fertilizer is not available or affordable; however, they are unlikely to be an exclusive N source for producing high yields. Therefore, an important aspect of their possible use is integrated nutrient management. Deep placement of N fertilizer decreases N losses by volatilization and permits photodependent N<sub>2</sub> fixation by cyanobacteria. Combining N deep placement with other practices that favor cyanobacteria growth is a good example of the kind of technology necessary to take advantage of the cyanobacteria potential in an optimized, integrated management of chemical fertilizers and photodependent N<sub>2</sub> fixation.

In-depth agroecological research is still required before cyanobacteria technology can be substantially improved.

### 7.5. Potential of N<sub>2</sub>-fixing photosynthetic bacteria associated with straw

Beneficial effects on BNF by the application of straw were first reported for heterotrophic microorganisms. Early estimates of BNF after straw incorporation range from 0.1 to 7 (mean 2.1) mg N/g straw added in 30 d (Roger and Watanabe 1986). Most data originate from laboratory incubations in the dark of soil enriched with 1-100% straw (average 22%), which simulates composting rather than the actual field situation where straw left is always less than 1% of soil dw.

It was then shown that straw also favors photodependent N<sub>2</sub> fixation (Barthakur et al 1983, Huang TanChi 1983, Roger et al 1982), even in acidic soils (Matsuguchi and Yoo 1981). Stimulatory effects of surface application of straw on cyanobacteria growth and photodependent ARA may be due to increased CO<sub>2</sub> availability, a decrease of mineral N and O<sub>2</sub> concentration in the floodwater, and the provision of microaerobic microsites by the straw. CO<sub>2</sub> availability and low N concentration favor the growth of N<sub>2</sub>-fixing cyanobacteria. Low O<sub>2</sub> concentration in the photic zone may increase their specific N<sub>2</sub>-fixing activity (Roger and Watanabe 1986). Quantitative estimates of BNF in field experiments with straw are not available, but a few semiquantitative data and laboratory data suggest that straw might increase BNF by 2-4 kg N/t of straw applied (Ladha and Bonkerd 1988).

On the other hand, surface application of straw might have a negative effect by favoring the spread of rice diseases (Mew and Rosales 1985) and the development of aquatic snails.

## 7.6. Rice-fish culture

Rice-fish culture<sup>1</sup> consists of stocking ricefields with fish fingerlings of a selected size and species to obtain a fish crop in addition to rice. Reviews, handbooks, and conference proceedings on rice-fish culture are available (Hora and Pillay 1962, Coche 1967, Huat and Tan 1980, Pullin and Shehadeh 1980, IIRR and ICLARM 1992, dela Cruz et al 1992).

### 7.6.1. Past and present status

The practice of collecting wild, naturally occurring fish for food from ricefields is probably as old as rice cultivation itself. Fields are usually not especially prepared for fish retention, but in some areas 40-50 m<sup>2</sup> sumps are established in the lowest region of a group of fields to facilitate collection and conservation of fish at the end of the rice-growing season.

Such fish-capture systems occupy a far greater area than cultural systems and are important in many rice-growing areas of Southeast Asia. In Bangladesh, an average 70 kg wild fish/ha is captured annually on the 2.8 million ha planted to rice (Dewan 1992). In Korea, where irrigated rice occupies 980,000 ha, about  $5 \times 10^4$  t of fish were captured and  $17 \times 10^4$  t were cultured in 1988 (a total 68 kg fish/ha of ricefield per yr) (Kim et al 1992). In Malaysia, the capture system was very successful after the introduction of *Trichogaster pectoralis* from Thailand into the rice-growing areas (Soong 1951). A recent strong decline in yield of captured fish in Malaysia (from 300-450 kg/ha per yr in the 1940s to 90-170 kg/ha per yr in the 1980s) indicates limitations of the capture method, especially in areas with double cropping where fields are flooded for a much shorter period (4 mo) than in single-cropped areas (6-8 mo). That, together with the extensive use of pesticides, has caused a rapid decrease in spontaneous fish production (Ali 1992). It may be necessary for farmers to change to cultural systems to maintain their income from fish.

The oldest written record of rice-fish culture is probably in a Chinese cook book from the third century, WeiWu's *Recipes of four seasons*, which indicates the suitability of a fish produced in ricefields—likely *Cyprinus carpio*—for making fish sauce (Li KangMin 1992).

Various methods of rice-fish culture have been used for centuries in India and Southeast Asia, however, rice-fish culture is very limited today. Potential and existing areas for rice-fish farming estimated by Lightfoot et al (1992) suggest that there are large areas suitable for rice-fish culture in many rice-

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<sup>1</sup>Three terms are used to describe the culture of rice and fish: 1) rice-fish (hyphen without spacing on either side), a generic term for rice and fish grown in association without regard to a specific cropping sequence, 2) rice + fish (spaced plus sign), a cropping pattern in which fish and rice are grown concurrently, and 3) rice - fish (spaced hyphen), a cropping pattern in which rice is followed by fish in rotation. (See sect. 7.6.2 for more complete description of rice - fish system.)

**Table 7.5. Potential and existing areas for rice-fish farming in Asia (adapted from Lightfoot et al 1992a).**

Country	Ricefield area <sup>a</sup>		Potential (ha x 10 <sup>3</sup> )	Existing (year) <sup>b</sup> (ha x 10 <sup>3</sup> )	Reference
	Total	Irrigated			
Bangladesh	10,229	1,227,615		Not known	Dewan 1992 <sup>c</sup>
China <sup>d</sup>	32,798	30,902	5000	985.5 (1986)	Xu and Guo 1992 <sup>c</sup> Li 1992 <sup>c</sup> , Wang 1992 <sup>c</sup>
India	40,991	14,347	2000	Not known	Ghosh 1992 <sup>c</sup>
Indonesia	9,889	6,230	1570	94.3 (1985)	Koesoemadinata and Costa-Pierce 1992 <sup>c</sup> , Syamsiah et al 1992 <sup>c</sup>
Japan	2,250	2,220	... <sup>e</sup>	13.9 (1943) 3.4 (1963)	Nambiar 1970 <sup>f</sup>
Korea, Republic of	1,229	1,118	127	0.1 (1989)	Kim et al 1992 <sup>c</sup>
Malaysia	647	427	120	45.5 (1962)	Hora and Pillay 1962 <sup>f</sup> Ali 1992 <sup>c</sup>
Philippines	3,426	1,473	181	1.4 (1982) 0.2 (1986)	Sevilleja 1992 <sup>c</sup>
Thailand	9,378	1,313	254	200.0 (1974) 2.8 (1983)	Rabanal 1974 <sup>f</sup> Fedoruk and Leelapatra 1992 <sup>c</sup>
Vietnam	5,691	2,276	326	1.5 (1960)	Hora and Pillay 1962 Mai et al 1992 <sup>c</sup>

<sup>a</sup> 1985-87 data from IRRI rice facts 1988. <sup>b</sup> Estimated area and year of estimation. <sup>c</sup> Cited by Lightfoot et al 1992a. <sup>d</sup> Excludes Taiwan Province. <sup>e</sup> No estimate available. <sup>f</sup> Cited by Huat and Tan 1980.

growing countries, but the current use is appreciable only in China and Indonesia (Table 7.5).

In China, where irrigated rice covers 31 million ha, rice-fish culture increased from 120,000 ha in four provinces in 1981 to almost 1 million ha in 17 provinces in 1986 (Li KangMin 1992).

In Indonesia, rice-fish culture started at the end of the 19th century. By the 1960s, it was practiced on approximately 50,000 ha with an average fish production of 100 kg/ha per yr. Currently rice - fish farming is reported in 17 of the 27 provinces of Indonesia. It was practiced on about 100,000 ha in 1984-85 with an average fish yield of 520-670 kg/ha per yr. The high average yield is due to the adoption of rotational cropping wherein a single crop of fish is stocked in ricefields after a single annual crop of rice has been harvested (Koesoemadinata and Costa-Pierce 1992).

In other countries, rice-fish culture is limited. The paucity of data in Table 7.5 also suggests that in many countries fish are cultivated in so few ricelands that national statistics rarely bother to record them.

### 7.6.2. Production techniques

Techniques for rice-fish culture differ considerably from country to country, and by region within a country, as observed in Indonesia by Ardiwinata (1957) and in China by Wang (1992).

In the most primitive rice-fish culture systems, fish were concentrated in the lower portion of the ricefields and were given some protection from predation by placing cut branches in deeper spots that were also used as a fish reservoir during DS (Heckman 1979).

In more advanced types of rice-fish culture, a refuge or canal is established to protect fish from field drying and to facilitate harvest. In some systems ridges are established, fingerlings are introduced at an appropriate time, and additional feed is often provided. Most sophisticated systems combine concurrent rice-fish culture with that of the N<sub>2</sub>-fixing *Azolla* used both as fish feed and as biofertilizer (Liu 1988).

The three major types of field design for rice-fish culture are 1) trench(es) within the ricefield, 2) a pond adjacent to the ricefield, or 3) a deepwater ricefield. Two types of growing periods are used, 1) fish concurrent with rice [rice + fish], and 2) rice followed by fish in rotation [rice - fish]. In the rice - fish system, fish are grown either as a single annual crop after rice or as an intermediate crop between the rice harvest and the next replanting.

Major species grown singly or in association are carp, tilapia, silver barb *Puntius gonionotus*, snakeskin gourami *P. javanicus*, and prawns. Although most systems are for growout operations, ricefields are also suitable for nursery operations.

Combinations of field designs, growing periods, and fish species result in a wide array of systems (Lightfoot et al 1992). Wang (1992) reported seven fish culture systems in China: 1) concurrent rice-fish fingerling production, 2) planting rice on ridges and culturing fish in ditches, 3) rice-fish-*Azolla* combination, 4) fish culture in ricefields with wide ditches, 5) fish culture in sumps and ricefield, 6) fish culture in winter-free or water reservoir fields, and 7) fish culture in low-lying ricefields.

7.6.2.1. *Production patterns.* The rice + fish system has a number of advantages because the fish help to control weeds, molluscs, and harmful insects and thereby increase rice yield. The dikes, drainage ditches, and capturing sumps take up between 5 and 10% of the field. The loss in space is usually compensated for by increased rice yields and added income from the fish. The rice + fish system requires some modifications of field design, adequate water inflow and depth, and rice varieties tolerant of deeper water. It does, however, limit the use of modern technologies or requires their adaptation, especially for application of chemical fertilizers and pesticides. The fish production cycle does not necessarily match the rice production cycle — it can be shorter or longer.

In Indonesia, the first stocking of common carp takes place 5 DT, at about  $6 \times 10^4$  1-cm fry/ha. At the second weeding, 5 wk after replanting, the fry (3-5

cm) are harvested and sold, and a second stocking is done with 1,000-2,000 fingerlings (8-10 cm)/ha to produce food fish. One and a half months later, fields are drained and fish of 14-16 cm, each weighing 50-70 g, are harvested. The yield varies between 75 and 100 kg/ha (Koesoemadinata and Costa-Pierce 1992).

In Western Java, *Sarotherodon mossambicus* is cultured simultaneously with rice. One week after transplanting, 1,000-10,000 fry (1-3 cm)/ha are stocked together with a few hundred adults. Six weeks later, the largest fish are harvested for consumption and the remainder returned to the field for another 6 wk.

In contrast, Japanese farmers culture common carp for 2-3 yr. The first year, fields are stocked with 3,000-6,000 fry/ha about 7 DT. Supplemental feed, which usually includes silkworm pupae, is given daily. Yearlings are harvested about 1 wk before rice and stocked in overwintering ponds until the next spring, when they are restocked for a second year to produce fish of marketable size. Some fish are reared for a third year until they reach a weight of more than 350 g (Kuronuma 1967).

The rice + fish method is currently used in most countries practicing rice-fish culture, but it is slowly being discarded in favor of the rice - fish method in a number of areas.

Rotation or rice - fish permits better care for both rice and fish. It allows the use of machinery, insecticides, and herbicides for rice production. It also allows greater water depth for fish production. Methods used in Indonesia were summarized by Koesoemadinata and Costa-Pierce (1992). The simplest method is a single fish culture season after a single rice crop per year. One or 2 wk after rice harvest, 50-cm-high bunds are built around each field and ditches 50 cm wide and 30 cm deep are dug around the perimeter of the field just inside the bunds. Bamboo pipes and screens are placed at the inlet and outlet to prevent the passage of fish and debris. Common carp is the main fish grown. Both fry and food fish are produced. While the fish are being cultured in the fields, the dikes, inlet, and outlet are inspected daily. During heavy rains the inlet is closed. About 2 wk after the stocking of fry, paddy shoots and other vegetation are removed. The entire process is repeated if there is a second fish crop. Predatory birds are scared away during the day.

Another method involves the cultivation of fish between two rice crops. The method originally aimed to produce food fish for the farmers, but as freshwater fish culture expanded, the fields were used mainly for the production of fry for which there is a ready market. The average yield is about  $15 \times 10^4$  5-8 cm fry, i.e., about 54 kg/ha. Part of the fry produced after the first month are restocked and grown to food-fish size.

Combinations of rotational and concurrent rice-fish systems arranged in a 1-yr sequential cropping pattern have been developed in Indonesia. Component systems described by Koesoemadinata and Costa-Pierce (1992) are the concurrent [rice + fish] (*minapadi*); fish culture in between two rice crops

[rice - fish - rice] (*penyelang*); and fish culture during long fallow period after the second rice crop [rice - rice - fish] (*palawija*). Examples of 1-yr cropping patterns are minapadi-minapadi-palawija; and minapadi-penyelang-minapadi-palawija. When practiced in combination, these systems can produce annual fish yields of more than 1 t/ha.

**7.6.2.2. Fish management. Types of production.** While most rice-fish production systems are growout operations, the ricefields can also be used for nursery operations. Ricefields are used for both in China, Indonesia, and Thailand (Lightfoot et al 1992a). Raising fingerlings in ricefields can be more profitable than growout, when there is a market for fingerlings, because shallow ricefields may be better suited to nursery systems (Costa-Pierce 1992). Indonesia and China have rice-fish culture systems for fingerlings integrated with inland aquaculture production networks. Rice farmers in Java produce common carp fingerlings for sale to fish farmers who grow the fingerlings to table size (Lightfoot et al 1992a).

**Suitable species for stocking.** Ruddle (1982) has reviewed the rice-associated, food-fish species. Table 7.6 lists fish species recorded in ricefields or used for rice-fish culture. Some are also cultured in fish ponds and storage reservoirs. Suitability of a species for ricefield culture requires tolerance for shallow water (average depth about 15 cm), high and variable temperatures (up to 34 °C with a 10 °C range), high turbidities, and low O<sub>2</sub>. Fish differ in their tolerance for high temperature. The swamp species *Ophicephalus striatus*, *Clarias batrachus*, and *Trichogaster* spp. are able to withstand higher temperatures than riverine fish. Asian varieties of common carp can tolerate up to 34 °C, provided that the O<sub>2</sub> content of the floodwater is sufficient, but their optimum temperature for culture is 22-28 °C. In the floodwater of ricefields stocked with fish, light penetration is reduced by high turbidity and photosynthesis is reduced. In general, tropical species can tolerate turbid waters, but prolonged exposure to turbid conditions favors the growth of olfactory feeders such as *Clarias* spp. over visual feeders. Oxygen availability is a major factor limiting fish growth and survival (Soong 1951). In tropical inland waters, the dissolved O<sub>2</sub> content is normally about 70% saturation and adequate for fish. Organic pollution, however, may drop that to dangerously low levels. The minimum level of dissolved O<sub>2</sub> varies with size and species. Anabantids and clarids possess auxiliary air-breathing organs which allow them to survive at very low levels of dissolved O<sub>2</sub>.

**Polyculture.** By stocking combinations of fish species with different feeding habits, all available niches can be exploited. In Indonesia, Ardiwinata (1957) reported stocking combinations involving four and five genera. Under polyculture, the choice of fish should maintain a balanced system and consider the predatory/prey relationship of species. The snakehead *Ophicephalus striatus* is a voracious predator and should not be stocked with its prey species.

**Table 7.6. Main fish species grown or found in ricefields.**

Scientific name	Common name	Countries of occurrence	Source
<i>Anabas testudineus</i>			Huat and Tan 1980
<i>Anguilla japonica</i>	Eel	India and Japan	Yoshihiro et al 1958 Anon 1956
<i>Cambarus clarkii</i>	Crayfish	Japan	Yoshihiro et al 1958
<i>Catla catla</i>			Huat and Tan 1980
<i>Carassius auratus</i>	Goldfish	Japan	Yoshihiro et al 1958 <sup>a</sup>
<i>Chanos chanos</i>	Milkfish	India and Indonesia	Menon 1954 <sup>a</sup> Djaingsastro 1957
<i>Cirrhina mrigala</i>			Huat and Tan 1980
<i>Clarias</i> spp.	Catfish	Malaysia and other Southeast Asian countries	Gopinath 1955 <sup>a</sup> Soong 1950 <sup>a</sup> Tonolli 1955 <sup>a</sup>
<i>Cyprinus carpio</i> and other species	Carp	China, Hungary, Indonesia, Italy, Japan, Madagascar, Pakistan, Spain, and some Southeast Asian countries	FAO 1957 <sup>a</sup> Coche 1967 Grover 1979 <sup>a</sup> Arce 1977 <sup>a</sup>
<i>Helostoma temmincki</i>			Huat and Tan 1980
<i>Labeo rohita</i>			Huat and Tan 1980
<i>Lates calcarifer</i>	Bhekti	India	Pillay and Bose 1957 <sup>a</sup>
<i>Mugil</i> spp.	Grey mullet	India and some isolated islands in Asia	Pillay and Bose 1957 <sup>a</sup>
<i>Mystus gulio</i>	Tengra	India	Pillay and Bose 1957 <sup>a</sup>
<i>Ophicephalus striatus</i>	Mudfish	India, Malaysia, Philippines, and Vietnam	Soong 1954 <sup>a</sup> Wyatt 1956 <sup>a</sup> Le Mare 1949 <sup>a</sup>
<i>Osteocheilus hasseltii</i>			Huat and Tan 1980
<i>Puntius goniotosus</i>			Huat and Tan 1980
<i>Sarotherodon mossambicus</i>	Tilapia	Indonesia; Taiwan, China; Philippines; and other Southeast Asian countries and Africa	FAO 1957 <sup>a</sup> Coche 1967 Grover 1979 <sup>a</sup> Vincke 1979 <sup>a</sup> Arce 1977 <sup>a</sup>
<i>Trichogaster pectoralis</i>			Huat and Tan 1980
<i>Trichogaster tricopterus</i>			Huat and Tan 1980

<sup>a</sup>Cited by Singh et al (1980).

For irrigated rice, rice-fish culture techniques using tilapia and carp are promising because they combine an omnivorous species with a mud feeder and optimize the exploitation of the food sources.

Yields from rice-fish culture are affected by the fish species composition. In experimental plots in China, highest rice yield was obtained with 30% grass carp (*Cyprinus idella*), 20% common carp, and 50% crucian carp (*Cyprinus*

*auratus*); higher fish yield was obtained with 50% grass carp, 30% common carp, and 20% crucian carp (Xu and Guo 1992).

**Size and density of stocking.** Most fish species grown in ricefields are prolific spawners, which aids stocking whether by active introduction or by passive introduction of eggs and fry from water sources. Active stocking is done by introducing selected *fish nests*, eggs, fish fry, and fingerlings, or male and female adults obtained from the farmer's own brood stock, government agencies, or from other fish farmers.

In parts of China, fish nests of the common carp are introduced into ricefields with suitable conditions for hatching (China Freshwater Fish Committee 1973). In Indonesia, the eggs of *Osphronemus gorami* are introduced into the fields and 8-11 cm fingerlings harvested and sold for stocking into other ponds. Male and female *Helostoma temmincki* are also introduced into the ricefields and, when the fry are produced, the young of common carp or *Osteochilus hasseltii* are added (Ardiwinata 1957). The size of fry or fingerlings used varies with species and site. Fry and fingerlings of the various carp species grown in Chinese ricefields range from 1 to 12 cm (China Freshwater Fish Committee 1973). *Puntius gunionotus* fingerlings measuring 5-8 cm and *Osphronemus gorami* fingerlings of 8-11 cm are introduced into ricefields in Indonesia (Ardiwinata 1957). Tilapia fingerlings of 9-12 cm are stocked in ricefields in China (Tapiador et al 1977). In Japan, common carp fry, yearlings, and 2-yr-olds have been stocked (Kuronuma 1967).

The size and density of stocking varies with the species and the type of production. Under the palawija method of Indonesia, to raise fry 3-5 cm long, 1-cm fry are stocked at  $6 \times 10^4$  to  $10 \times 10^4$ /ha and grown for 3 wk. To raise fry 5-8 cm long, 2-3-cm fry are stocked at  $2 \times 10^4$ /ha and grown for 3-4 wk. To grow food fish, 5-8-cm fry from the previous growing period are stocked at 6,000/ha and grown for about 40 d, producing fish of about 100 g (Koesoemadinata and Costa-Pierce 1992).

The introduction of fish in a ricefield requires modifications of the field design and rice cultivation methods including land preparation; water requirement; planting method; fertilizer application method and fertilizer requirement; pest, disease, and weed control; harvesting method; and land management between crops.

*7.6.2.3. Modifications of the ricefield design for rice-fisk culture.* Growing fish in ricefields demands greater water depth. Bunds surrounding the field must be raised to allow maintaining required water depth, which depends on the size and type of fish cultured. Depths of up to 60 cm are used in India, but in most countries water depth is maintained between 15 and 20 cm. For the culture of tilapia, 7.5 cm of water is sufficient. Strong, watertight bunds are essential for the retention of water and to prevent rats from boring holes (Hora and Pillay 1962).

The water inlet and outlet—installed diagonally opposite each other to enable even water distribution—are screened to prevent the loss of fish and

the entry of undesirable species. A screened spillway is installed at a suitable height in the field to control the water level during heavy rains.

In general, three types of field design predominate: 1) shallow trench within the ricefield in China, Indonesia, and the Philippines; 2) pond refuge adjacent to the ricefield in China, India, Indonesia, and Thailand; and 3) deepwater ricefields in Bangladesh and India (Lightfoot et al 1992a). Channels are dug, usually on the inner sides of the bunds, to provide a retreat for fish during temperature extremes or during agrochemical applications. The channel is usually 50 cm wide and about 30 cm deep. If a field is less than 0.5 ha, a channel around half the perimeter or along two sides of a square field is sufficient. In larger fields, channels are peripheral or crosswise. A sump, approximately 1 m<sup>2</sup> in area, is sometimes dug at points where the channels meet to provide a retreat for fish and to aid harvesting (Hora and Pillay 1962). Pond refuges adjacent to the planted area are deeper than the trenches (50-100 cm) and occupy 5-7% of the field. In some cases, they are connected by a canal to the ricefield. Li KangMin (1992) reported ridge-ditch field designs with rice-*Azolla*-fish systems. Similar methods are used in Indonesia, Malaysia, the Philippines, and Thailand (dela Cruz et al 1992). The ridge-ditch design is similar to the Indonesian *surjan* design (Koesoemadinata and Costa-Pierce 1992) although the latter has much wider ridges and ditches.

**7.6.2.4. Modifications of rice agronomy for rice-fish culture. Rice varieties and planting method.** Optimum water depth for fish (15-25 cm) is deeper than the 10 cm optimum for many modern varieties (MVs) of rice. Such varieties have shorter growth duration (95-125 d) than most traditional varieties (160 d). Fish stocked in fields planted with MVs must be able to grow to a harvestable size within 80-100 d or must be held in refuges or ponds during rice harvest and until the next crop is planted.

Traditional rice transplanting at 20 cm between rows and 15 or 20 cm between plants allows enough space for fish movement. Space for fish can be increased while keeping the same plant density by increasing the distance between rows to 25-30 cm and reducing the distance between plants.

To avoid fish damage to the rice plants, fish should not be stocked until 10 DT when fry are used and 21 DT when fingerlings are used. When seedlings germinated without soil (*dapog*) are used or direct seeding is practiced, fish must be stocked even later to avoid damage to the young seedlings (Singh et al 1980).

**Water management.** The water requirement in ricefields where fish are stocked may double or even triple because at least 10 cm water is required in the planted area and 30-60 cm in trenches and refuges. That may result in a water shortage for the succeeding crop. Water change or leakage should be avoided as it affects the general fertility of the field and the production of phytoplankton and zooplankton used for food by fish. That suggests that fish should be grown in areas where water supply is abundant and soil percolation is low. Intermittent irrigation, practiced in some rice-growing areas, is

possible if trenches or refuges are kept full of water during dry periods. Extending the fish culture period beyond that of rice, or growing fish over successive crops of rice, requires increased management of irrigation systems and water delivery to the fields between crops.

**Fertilizer management.** To enhance primary production and associated fish food chains, 50-100% more fertilizer is required for rice-fish culture than for rice alone (Chen 1954).

Most farmers broadcast N fertilizer in the floodwater in two or three split applications. Large amounts and untimely broadcasting of fertilizers may suppress fish growth due to high  $\text{NH}_3$  concentrations in water (Colt and Tchobanoglous 1978). Applying N fertilizer in split doses to achieve maximum use by the crop and to avoid high N concentrations in floodwater is recommended. When fertilizer is broadcast later in the crop cycle, floodwater should be drained to expose the planted area, and fish should be confined to the trenches by draining sufficient water from the field (Singh et al 1980). The advantages of deep placement of N fertilizer, in terms of N use efficiency and environmental conservation, are well-known (Roger et al 1980, Simpson and Roger 1991). Inasmuch as deep placement reduces the growth of algae and algal grazers, it might be less favorable for fish production than broadcasting.

Phosphorus application is important for rice production and the growth of phytoplankton and zooplankton. Surface application and soil incorporation produce similar rice yields, but split broadcasting before tillering may prove better for sustained plankton growth.

Organic manures are suitable for both rice and fish. They can act as a substrate for the growth of fish food organisms. To avoid any toxic effects, however, they should be applied several weeks before transplanting and the fields should be kept flooded to achieve complete decomposition (Singh et al 1980).

**Pesticide use.** Several authors attributed the decline in rice-fish farming to pesticide use (de la Cruz et al 1992). Irrigated ricefields, which are most suited for aquaculture, have been the most affected because they are treated with more pesticides than rainfed and deepwater ricefields. Tabulated data on the toxicity to fish of pesticides used in ricefields are found in Liu (1988), Li KangMin 1992, Xiao (1992), Koesoemadinata and Costa-Pierce (1992), and Cagauan and Arce (1992). Major groups of pesticides ranked from the most to least toxic to fish are insecticides, molluscicides, and herbicides in that order. Carbamate and organophosphate insecticides are less toxic to fish than organochlorides and synthetic pyrethroids (Cagauan and Arce 1992).

Proper application methods and selection of chemicals can alleviate toxicity problems (Kim et al 1992, Koesoemadinata and Costa-Pierce 1992, Sevilleja 1992, Xiao 1992). Suitability of pesticides for rice-fish culture depends mainly on their formulation, persistence, and the method and time of application before fish are released into the field. Cagauan and Arce (1992) reported how acute toxicity data generated from laboratory bioassay can be extrapolated

for selection and determination of adequate concentrations for field use. Heinrichs et al (1977) indicated that, ideally, fish should be removed from the field at least for 10 d if insecticides are to be sprayed or broadcast. Alternatively, the fish can be confined to the trenches by lowering the water level, but no runoff water should be allowed into trenches for at least 1 wk after reflooding the field. That could be done by increasing the height of the trench dikes and incorporating pesticides into the soil. According to Cagauan and Arce (1992), application of granular forms of pesticides 1 wk before fish introduction was sufficient for degradation of the chemicals and resulted in no fish mortality. Generally, residues of commonly applied insecticides were not detectable in water or in fish tissues at 7 d after application in ricefields.

There is increasing evidence that economic benefits from insecticide use under farmers' conditions are less than previously claimed. Litsinger (1984) estimated that rice production in the Philippines could be maintained with about half of the present level of insecticide use. On-farm trials showed that farmers' pesticide applications paid off in less than 50% of the cases in the Philippines and incurred a loss of \$3.70/ha in Thailand (Waibel 1992). As a result, insecticide use is decreasing in some countries, while pest-resistant varieties and integrated pest management (IPM) are being adopted. One advantage of introducing fish into ricefields is that farmers must then consider the economic threshold of pesticide use and tend to use less.

**Harvesting.** Ricefields are usually drained 1-2 wk before harvest to facilitate harvesting. Fish should be harvested at that time or confined to the refuges.

### 7.6.3. Economics

7.6.3.1. *Fish yield.* Fish yields from the capture system listed by Huat and Tan (1980) are 100-200 kg/ha per yr in India, 1.5-3.0 kg/ha per yr in Indonesia, and 10-400 kg/ha per 6-10 mo in Malaysia.

Under the culture system, fish yield depends on the species stocked, the culture period, the fertility of the soil and water, and the degree of supplemental feeding. Soong (1951) estimated that in Malaysia, yield was between 220 and 450 kg/ha with neutral water, and 11-56 kg/ha with acidic water. According to Ardiwinata (1957), under rotational culture, the production of food fish was about 600 kg/ha in fertile waters, 300 kg/ha in moderately fertile waters, and 100-200 kg/ha in infertile waters. For common carp fry culture, production of 4- to 5-cm fry was about  $10 \times 10^4$  to  $20 \times 10^4$ /ha, together with about 50-100 kg of food fish/ha. For fish grown between two rice crops, the yield was 40-60 kg/ha;  $4 \times 10^4$ - $6 \times 10^4$  3-5-cm fry/ha, or  $2 \times 10^4$ - $3 \times 10^4$  5-8-cm fry/ha, accompanied in each case by an additional 20-30 kg of food fish/ha. Farmers culturing *Puntius gonionotus* produced about  $8 \times 10^4$ - $10 \times 10^4$  2-3-cm fry/ha under the same system.

Concurrent rice-common carp culture yielded about 50-70 kg food fish/ha. The corresponding yield for *P. gonionotus* was 40-60 kg/ha and for both fish stocked together, about 60-100 kg/ha.

**Table 7.7. Ranges of recorded effects on grain yield of fish stocking in ricefields.**

Yield change (%)	Country	Reference <sup>a</sup>
+ 4.6 to 28.6	Several (review)	Lightfoot et al 1992a
+ 14	Bangladesh	Haroon et al 1992
+ 11 to 35	China <sup>b</sup>	Nie et al 1992
+ 1 to 11	India	Mukhopadhyay et al 1992
+ 9 to 23	India	Ghosh 1992
-2	India	Mukhopadhyay et al 1992
+ 6.6	Indonesia	Syamsiah et al 1992
= or > 0	Korea, Republic of	Kim et al 1992
None	Philippines	Torres et al 1992
+ 47 to 50	Philippines	Fermin 1992
-29 to -1 in 9 of 25 farms	Thailand	Thongpan et al 1992
> 0 in 16 of 25 farms	Thailand	Thongpan et al 1992

<sup>a</sup>All references cited by dela Cruz et al 1992. <sup>b</sup>Excluding Taiwan Province.

7.6.3.2. *Rice yield and comparison with monocropping.* Even though rice-fish farming has been practiced in Asia for many years, little quantitative data on rice yield effects exist. Lightfoot et al (1992b) concluded from a bibliographic compilation that the study of fish effects on rice is replete with hearsay and confounding, inconclusive experiments. The analysis of the data from 18 studies in China, India, Indonesia, the Philippines, and Thailand showed both yield increase and decrease. The range of differences was very wide (-58% to +183%). Differences, however, strongly favored positive outcomes, with an average 15% increase in rice yields calculated on the basis of the area planted to rice (Table 7.7), a value similar to that reported earlier by Ruddle (1982).

Lightfoot et al (1992b) also indicated that in the work they surveyed, little explanation of why yields were high or low was found except in cases where crops were severely damaged. Experiments where rice yields from rice - fish and rice alone were compared on the same farm indicated that growth and production of the individual rice plants increased in rice-fish culture. Spiller (1985) indicated that rice yield increased as fish production increased.

7.6.3.3. *Production budget.* One of the traditional additional sources of income for rice farmers has been the sale of fish harvested under the capture system. Hickling (1961) estimated that fish provide an additional income, varying from 20 to 33% of the income from rice culture.

Greater input and management requirements of rice-fish culture create added costs for the farmer. They include the cost of 1) physical modifications made to the ricefield—if hired labor is employed, 2) fish fry or fingerlings, 3) supplemental feed, and 4) additional fertilizer. In Indonesia, Ardiwinata (1957) estimated that the cost of fry was about 30-50% of the value of the fish crop, while maintenance expenses accounted for 5-20%, leaving a profit margin of 30-65% of the value of the fish cropped.

An economic analysis in the Philippines showed that a shift from rice monoculture to rice-fish culture required 17% more labor and 22% more working capital, but the additional fish production generated 67% more farm income (Ahmed et al 1992). In China, increases in gross return by 30% for rice-fish culture and by 50-60% for rice- *Azolla* -fish culture were calculated for both single and double rice cropping systems (Hunan Research Team 1987).

Intensive rice-fish nursery systems, as described by Costa-Pierce (1992) in Indonesia, can be very profitable because of the demand for fingerlings for restocking. A rotational system studied for 1 yr produced 11.7 t of rice from 2 crops and 791 kg of fish seed from 5 crops. Net return was about \$1,700 with fish making up 31% of the total.

The relative income from rice and fish varies markedly between owner farmers and tenant farmers and between single cropping and double cropping. In Malaysia, when a single rice crop was grown, the income from fish, expressed as percentage of income from rice, averaged 25% for owner farmers and 50% for tenant farmers. Experienced tenant farmers cede the entire rice crop to landowners in exchange for the right to culture fish in the ricefields (Huat and Tan 1980).

#### **7.6.4. Agroecological interactions in rice-fish culture**

Besides proteinous food production, fish culture in ricefields may have several beneficial effects, which include 1) control of rice pests—insects, weeds, and snails; 2) improvement of physicochemical properties of soil; 3) possible reduction of N losses from water due to NH<sub>3</sub> volatilization; 4) increase in nutrient cycling and availability; and 5) increase in rice yield and N uptake (Lightfoot et al 1990).

Many mechanisms have been suggested to explain the yield increases observed in ricefields stocked with fish. Experimental evidence to support these hypothesis, however, is not only meager but often dates back to the 1950s and 1960s. Coche's often quoted weed effect is based on experiments in Africa in the 1950s (Coche 1967). Many of the still unquantified mechanisms were put forward by Ardiwinata (1957).

7.6.4.1. *Indirect effects.* Some effects are clearly indirect rather than direct. The additional tillage and precise water control necessary for good fish production will, through better weed and nutrient management, increase rice yields, whether or not fish are present.

7.6.4.2. *Effects on soil fertility and nutrient availability.* Various pathways have been traced for the contribution of fish to soil fertility (Huat and Tan 1980, Lightfoot et al 1992b). Introducing fish into ricefields may improve the fertility of the ecosystem by increasing nutrient cycling and availability to the rice plant, improving soil structure, and decreasing N losses.

Fish grazing on the aquatic biomass contribute, through their feces, to increased nutrient recycling and availability to rice. Organic matter, N, available phosphorus, and K were all higher in fields where rice had been grown

with fish compared with fields of rice alone (Hunan Research Team 1987). Differences were most marked in the soils of the sumps and trenches where fish preferentially stay. These data, however, were presented without statistical analysis.

An increase in rice yield after stocking phytoplankton feeders was attributed to fish grazing on the algae covering the surface of the soil and to better nutrient recycling (Cagauan 1991). That agrees with the observation that fish stocking increased total N yield of rice. Increases of N concentration in rice grain by 5% and N uptake by 10% were observed in fields where fish were introduced (Panda et al 1987).

Fish stocking also increased P availability (Satari 1962). At the same level of triple superphosphate (100 kg/ha), rice yield in a rice-fish system was 1.3 t/ha higher than that in the control with no fish (Fagi et al 1992). Increased P uptake by rice in the presence of fish was also reported from experiments in concrete tanks but differences were not significant in earthen tanks (Panda et al 1987).

Iron uptake by rice was two times higher in rice-fish culture than in rice alone (Panda et al 1987).

Grazing reduces algal biomass and increases water turbidity. That helps keep the pH near neutral, which in turn reduces N loss through  $\text{NH}_3$  volatilization in the earlier stages of the crop. The potential of fish to reduce to some extent N loss from the water through volatilization was demonstrated by a pH significantly lower (0.3-0.6 units) in fields with fish compared with a control with no fish (Sevilleja and Cagauan 1992).

7.6.4.3. *Interactions between fish and rice pests.* Of the approximately 100 insect species and 74 diseases and physiological disorders associated with rice (Teng et al 1990), 30 insects and 16 diseases are considered economically important (Riessig et al 1986). Generalized crop loss figures most commonly cited are those by Cramer (1967), who concluded that more of the rice potential production is lost due to pests (55%) than is harvested (45%). He estimated the percentage of potential harvest lost due to pests to be 34% for insects, 10% for diseases, and 11% for weeds. Although these figures appear to be high at first glance, Teng et al (1990) found that other authors have reported no good evidence to the contrary.

**Pests.** Several reports from China indicate that concurrent fish culture reduces rice insect pests. Estimates of pest reduction by fish consumption range from 12 to 75% (Liao 1980). Some farmers drag a rope across the rice canopy to knock insects into the water for fish to feed on. Liu (1988) reported reductions in rice leafhoppers by 45-60% in rice-fish culture as compared with rice monocropping. Interestingly, fish seemed not to prey on spiders. Other work has found fish to be predators of rice stem borers, rice leafhoppers, and rice planthoppers (China Freshwater Fish Committee 1973, Spiller 1985). Xiao (1992) presents sets of data showing numerically lower populations of leafhoppers, stem borers, and planthoppers in ricefields with fish as compared

with fields without fish. Unfortunately, none of these reports present statistical analysis of the data.

Rat infestation can also be greatly reduced by the higher and more solid bunds erected for rice-fish culture.

**Diseases.** Sclerotia of sheath blight (Xiao 1992), brown spot, and bacterial blight (Halwart 1991) were reportedly controlled by fish. In China, Liu (1988) reported a 75% reduction in rice stripe disease incidence in rice-fish and rice-fish-*Azolla* culture, and a reduction by 70% in rice-*Azolla* culture, compared with rice monocropping. The mechanism for disease control is not clear. Tilapia and common carp in ricefields commonly eat detritus which contains pathogens, but the possible effect on disease incidence has not been studied.

**Weeds.** Herbivorous fishes such as grass carp, Zill's tilapia, and silver barb directly consume weeds. Bottom feeders such as the common carp may indirectly reduce weed incidence by uprooting weeds and increasing water turbidity, which reduces light availability for photosynthesis. Inasmuch as many weeds cannot germinate in flooded fields, the reduction of weed growth in fields where fish are cultured may be due partly to the increased water depth (Moody 1992).

Fish-weed interactions were reviewed by Moody (1992). Cagauan (1991) listed 12 investigations demonstrating the potential of various fish species for controlling rice weeds. Marked reduction of weed density and weed fresh weight in fields where fish were stocked was reported in China (Liu 1988, Xu and Guo 1992).

For the last two decades, research has focused on the potential of grass carp for aquatic weed control as an alternative to conventional manual, mechanical, and chemical methods (Van Zon 1980, cited by Cagauan 1991). Grass carp feed on floating weeds and submerged weeds such as *Hydrilla* and *Eleocharis* (Nie DasHu et al 1992). Floating weeds disappeared after 1.5 mo, then submerged species, after 30 g common carp were stocked in ricefields at 1,000-6,000/ha (Tsuchiya 1979). Nie DasHu et al (1992) reported weed biomass decreased by 14-15 times in fields where grass carp were stocked at 2/m<sup>2</sup>, compared with a control without fish. In Indonesia, weed production was reduced by 40-47% after mixed stocking of common carp and tilapia (Satari 1962).

Many other herbivorous fish can reduce weed incidence. Among the most useful species are *Puntius gonionotus*, *Sarothrodon mossambicus*, *Trichogaster pectoralis*, and *Cyprinus carpio*. Stocking *Tilapia melanopleura* at 7 adults/ha in experimental ricefields in Katanga 3 wk after transplanting kept ricefields free of weeds and algae (Coche 1967).

But not all fish reduce weed incidence. In ricefields of the Philippines, *Hydrilla* grew abundantly in fields stocked only with Nile tilapia *Oreochromis niloticus*, but weeds and algae were relatively absent when fields were stocked with a mixed population of Nile tilapia and common carp (dela Cruz 1980). Satari (1962) reported that the culture of fish before transplanting rice stimu-

lated the growth of phytoplankton, especially cyanobacteria. That effect might be related to an increased P availability through fish feces.

7.6.4.4. *Interactions between fish and vectors of human diseases.* Fish in ricefields are known to consume some of the intermediate hosts and vectors for water-borne diseases, especially mosquito larvae and snails. A grass carp fingerling can eat 272 mosquito larvae in 24 h. Stocked at 2/m<sup>2</sup>, fingerlings can almost eliminate mosquito larvae in the ricefield (Nie DasHu et al 1992). After a 3-yr experiment in Africa, Coche (1967) concluded that the majority of snails normally present in the ricefield could be controlled by the joint action of *Tilapia melanopleura* and *Haplochromis mellandi* eating snail. Common carp and Nile tilapia also consume newly hatched snail (*Pomacea* sp.), a serious rice pest in the Philippines (Halwart 1991). The overall fish consumption of vectors and intermediate hosts of human water-borne diseases, under different conditions of food abundance and diversity, remains to be studied. Likewise the contribution of stocked fish to lessening the prevalence of vector-borne disease remains to be quantified. Fish in ricefields, however, can be expected to lessen rather than exacerbate human disease problems.

7.6.4.5. *Detrimental effects of fish.* Stocking large herbivorous fishes may damage rice plants. There have been reports of carp uprooting rice seedlings if stocked too early (Khoo and Tan 1980). Stocking the fish when rice seedlings are well established prevents such damage (Tsuchiya 1979). Diverting the attention of grass carp by feeding grass when rice seedlings are young is another way. Stocking Nile tilapia negatively affected rice yields in experiments in the Philippines (Van Dam 1990).

Oligochaetes in ricefield floodwater that may have beneficial effects on rice were reduced as a result of fish grazing and pesticide toxicity (Sevilleja and Cagauan 1992); data are too fragmentary, however, to draw definite conclusions.

## 7.6.5. Prospects

Rice-fish systems presently occupy only a very small percentage of the potential area (Table 7.5), and there is a large scope for expansion. Pillay (1973) estimated that if only 30% of the then existing 35.6 million ha of irrigated rice were used for fish culture, even at a very low rate of production, an annual yield of about 2.2 million t of fish would result.

In countries such as Indonesia, where the capture system has been traditionally practiced, there is a high potential to upgrade to the rice-fish culture system.

Culturing fish is not traditional in irrigated and deepwater environments. In the recent past, rice-fish systems for irrigated (China, Indonesia), rainfed (Thailand), and deepwater environments (Bangladesh, India) have been pioneered generally by farmers themselves. But now agricultural research is involved in developing improved systems for these environments. Interest in deepwater rice-fish farming is growing in Bangladesh and India, which have

about 5.5 million ha of deepwater rice areas. Rice-fish culture techniques for irrigated rice using tilapia and carp are promising but have not yet diffused to many farmers. Most production systems are growout operations, but ricefields can also be used economically for nursery operations.

Major technical constraints to fish stocking in ricefields are 1) additional work, 2) shortages of fry or fingerlings, 3) negative effects on rice cultural practices, 4) detrimental effects of pesticides on fish, and 5) possible detrimental effects of fish on rice and useful fauna.

In particular, the prospects for concurrent culture may be restricted because of indiscriminate use of insecticides. But with the increasing awareness of the effects of some insecticides on both fish and the ecological balance between insect pests and predators, there is an increasing emphasis on biological control methods and less use of harmful pesticides. There is evidence that, with some exceptions, pesticides do not necessarily restrict the adoption of rice-fish culture. Research on rice-fish culture is also directed toward its supportive role in the expansion of IPM, which could act as a vanguard for expansion in rice-fish culture (Lightfoot 1992).

Because of economic advantages, rotational culture will probably become more important in the future.

Rice-fish culture, either concurrent or rotational, offers a promising alternative to rice monocropping by diversifying production, offering new opportunities for farmers to supplement their income, ensuring food security, enforcing sound use of agrochemicals, and favoring IPM and biodiversity conservation.

## 7.7. Other types of aquaculture in ricefields

Many edible animal species other than fish (e.g., shrimp, prawns, frogs, snails, crayfish) are found in ricefields. In deepwater ricefields, various species of freshwater shrimp constitute a significant part of the harvest from the capture system. Farmers use different kinds of traps to catch shrimp and crabs (Mukhopadhyay et al 1992). In the traditional system in brackish water areas of Bangladesh, ricefields are enclosed by embankments with inlet and outlet channels controlled by sluice gates. Tidal water carrying shrimp post larvae (*Penaeus* spp. and *Metapenaeus monoceros*) and fish juveniles are trapped in the field, which may produce about 200 kg of shrimp and 80 kg of fish/ha (Haroon et al 1992).

In deepwater and coastal areas of Bangladesh, India, and Vietnam, shrimp are added to the rice-fish systems. Giant freshwater prawns (*Macrobrachium rosenbergii*) and finfishes are stocked during wet or monsoon months; marine shrimp and fish are cultured during summer or saline water months. Marine shrimp (*Penaeus monodon*) can be cultured in fresh water/brackish water fields after a 10-d acclimatization period (Koesoemadinata and Costa-Pierce 1992). Trials of mixed culture of silver carp and giant freshwater prawn in a sewage-

treated deepwater ricefield produced 1.7 t rice, 500 kg of prawns, and 200 kg of fish/ha with a fish culture period extended to 8 mo (Mukhopadhyay et al 1992). In brackish areas of Bangladesh, rice-shrimp culture trials with different levels of fertilizer application for rice yielded between 160 and 390 kg freshwater giant prawn/ha within 180-240 d. In the best case, more than 80% of the prawns had a harvested weight > 30 g, up from an initial weight of about 8 g (Haroon et al 1992). Rice-shrimp culture is also developing in China (Li KangMin 1992).

Crayfish (*Procambarus clarkii*) can also be grown in ricefields. A study in Louisiana, USA (Yew and Avault 1980), showed that harvestable (>70 mm) crayfish production was higher (1.1 t/ha) in planted fields than in controls without rice (0.8 t/ha), partly due to a larger size of animals in the presence of rice. A positive correlation was observed between rice yields and the number of adult crayfish present during the rice-growing season. Stocking rate had little effect on crayfish production in the experimental fields where crayfish were already present. The authors indicated that in this area stocking is considered necessary only in new ponds.

In China, Li KangMin (1992) reported trials of a rice + fish + frog culture as an integrated pesticide - free system in which fish and frogs control rice pests and diseases and are harvested as food.

## 7.8. Control of detrimental fauna

Floodwater fauna in ricefields can be detrimental by their effects on the rice plant itself or as vectors of human and animal diseases (see sect. 5.5). Their effect as disease vectors is far more important, both sociologically and economically.

### 7.8.1. Vector control

The control of vectors of human and animal diseases that develop in ricefields can be achieved, at least partly, by water management, certain agricultural practices, pesticide application—including botanical pesticides, and biological control.

7.8.1.1. *Water management.* Methods of water management for controlling vectors in ricefields include 1) alternate wetting and drying, 2) maintaining soil saturation, and 3) quick flushing. Of the three, the alternate wetting and drying method seems most promising.

**Maintaining soil saturation.** Maintaining soil saturation, which avoids stagnant water on the soil, is a water-efficient method of rice production that provides vector control, but it is technically difficult to achieve. It requires fields that remain well leveled throughout the season; farmers' activities in the field leave many small depressions and can negate the effort. Constant soil saturation also requires precise and frequent applications of irrigation

water, which is almost impossible to achieve from a practical standpoint (Bhuiyan and Shepard 1987).

**Quick flushing.** The periodic quick flushing method to remove the vector larvae, described by Amerasinghe (1987), also suffers from the requirement of well-leveled fields free of depressions, and water control infrastructure for effective flushing. Furthermore, flushing requires substantial extra water, which cannot be obtained from most irrigation systems during DS when water is in short supply.

**Alternate wetting and drying.** Alternate wetting and drying of fields during the cropping season has been recommended to control hemipterous pests such as the brown planthopper *Nilaparvata lugens* (FAO/UNEP 1982). Those procedures are equivalent to those recommended for vector control based on alternate wetting and drying (Mather and Trinh 1984, FAO 1987, Lu Baolin 1988). Alternating submerged and dried phases must be such that mosquito vectors will be either flushed from the field or dried out before successfully completing immature development (Amerasinghe 1987), a period that can be as short as 5 d for *Simulium* spp. (Cairncross and Feachem 1983).

The alternate wetting and drying method of water management requires 30-50% less irrigation water than the conventional method of maintaining shallow (5-7 cm) submergence throughout the season (Bhuiyan et al 1988), if submergence water is allowed to dry up. The saving in water results mostly from reduced percolation losses. If, however, water from the ricefield is regularly drained off rather than used up to create the dry condition, the total water requirement would be higher and might even exceed that needed for conventional, continuous shallow submergence.

The alternate wetting and drying method will have a better chance of farmer acceptance if it is integrated with other measures for water conservation and vector control, assuming no negative effect on yield. Reports on the effects of such an irrigation method on yield are contradictory. Hill and Cambournac (1941) described a field trial of intermittent irrigation of ricefields that showed 1) rice quality and quantity did not suffer, 2) there was usually some yield increase and considerable savings in water, and 3) anopheline larvae were reduced by more than 80%. Some other reports show a yield increase (Cairncross and Feachem 1983, Luh 1984, Self and De Datta 1988), still others show a decrease (IRRI 1987a, IMMI 1986), especially under drying periods of more than 5 d. The reports on yield loss are mostly from areas in the Asian tropics with relatively low fertilizer use and high temperature regimes. Such conditions reduce the production of toxic substances in the soil, hence benefits from drying are low or nonexistent.

Alternate wetting and drying carried out in large areas of Hunan Province in East China reportedly conserved water, increased yield, and resulted in reduced densities of *Anopheles maculatus* and *Culex*, vectors of Japanese encephalitis (Self 1987). Field trials have shown the practice to control both snails and mosquitoes (Amerasinghe 1987). In the Philippines, replacing con-

tinuous flooding by alternate wetting and drying on a 10-d rotation regime reduced snails from 200/m<sup>2</sup> to less than 1/m<sup>2</sup> (Cairncross and Feachem 1983). A 90% reduction in mosquito larvae was reported in China when the method was applied in experimental fields (Luh 1984).

For optimum control, alternate wetting and drying must be practiced simultaneously for all ricefields in a large area, during the entire cultivation season, and under conditions favorable to rapid drying (Amerasinghe 1987). That requires a highly organized system of water application and strict adherence to the designed schedule. A major practical limitation of the method in most irrigation systems is the unreliable supply of irrigation water, which introduces the additional risk of drought damage to fields that are drained for vector control (Bhuiyan and Shepard 1987).

The method is not efficient for control of all vectors. For example, the operculum of *Oncomelania* enables it to survive for more than 1 wk without surface water (Garcia 1988), and many gastropod species undergo prolonged estivation and hibernation. Draining and drying the ricefield may adversely affect nontarget organisms, including aquatic predators.

**Importance of system-level irrigation management.** Vectors are not restricted to the ricefield. Water management in the ricefields will be of limited use unless irrigation system management is modified to control vectors that breed outside the ricefields, but within the system's command. Irrigation systems provide breeding habitats of snails and mosquitoes in canals with vegetation; stagnant pools created in depressed areas due to seepage from unlined canals; and dead storage in canals, borrow pits, choked drainage ditches, etc. Speelman and van den Top (1986) identified specific breeding sites of mosquitoes in a Sri Lankan irrigation system. Parts of the irrigation system outside the ricefields are considered the most important breeding grounds of schistosome snails. More farmers probably become infected while washing in irrigation canals than while working in ricefields (Garcia 1988).

Most snails feed on vegetation. There is a direct correlation between snail populations and the density of aquatic vegetation; therefore, feeder canals should be free of weeds to prevent explosive growth in snail populations. The weed provides not only food for the snails but also an egg-laying surface and cover for the hatchlings (Gaddal 1988).

**Crop intensification with crop rotation (wetland-dryland).** Crop intensification and crop rotation make possible irrigation practices that could prevent the breeding of snails, especially amphibious species. Where *Oncomelania* is prevalent, crop rotation can control or even eradicate snails (Pesigan et al 1958). Crop rotation effectively controls aquatic snails only where both the water distribution channels and the fields can be dried (Webbe 1988).

7.8.1.2. *Other agricultural practices.* **Mechanization and new implements.** As pointed out by Service (1977), very little information is available on the effects of mechanization on vector populations. Available data suggest that plowing before flooding (Owens et al 1970) and plowing followed by disking

(Cooney et al 1981) could control floodwater mosquitoes by burying mosquito eggs. Increased plowing or disking of ricefields can make the habitat unsuitable for snails (Ito 1970). Use of a rotary hydrotiller on a soil with about 1 cm standing water has reduced populations of large snails (*P. canaliculata*) by at least 50% (H. T. Manaligod and G. R. Quick, IRRI, Manila, Philippines, 1990, pers. commun.).

**Direct seeded rice.** In DSR, mosquito reproduction begins only when the established seedlings are flooded and peaks during the 4-wk postflooding period (Mather and Trinh 1984). Closure of the canopy takes place earlier than in transplanted rice. Implications for vector habitat have not yet been assessed.

**Synchronous planting and harvesting.** A mosaic of fields planted at different times provides a continuous sequence of crops for pest buildup. Synchronous planting and harvesting in large areas are highlighted as a means of creating off-season areawide breaks in cropping, which disturb the life cycle of rice pests (Perfect 1986). Synchronous planting with large areas under periodic fallow, unirrigated phases will also upset the breeding sequence of vectors (Way 1987). From a practical standpoint, however, synchrony is difficult to achieve in communities of small farm holdings, particularly because water availability is often asynchronous, and farmers are not ready with other inputs to start the crop all at the same time. Because MVs are photoperiod-insensitive, they allow individual timing of planting, which acts against the promotion of synchronous planting.

**Weed control and flood fallow.** Weed control in ricefields and related water bodies can sometimes reduce the distribution and abundance of intermediate snail hosts of schistosomiasis and mosquito vectors. For example, certain species of *Potamogeton* provide good surfaces for feeding and egg laying by *Biomphalaria truncatus* and *B. alexandria* (WHO 1973). Algae, combined with decaying vascular plants, generally provide the best food for snail hosts. Several aquatic weeds, such as *Pistia* and *Salvinia*, furnish highly favorable habitats for *Mansonia* mosquitoes. Often these weeds are not so much in the ricefields but in the irrigation channels, ditches, and water reservoirs.

Repeated plowing, harrowing, and weed incorporation render ricefields less suitable for snail and mosquito populations. However, some mosquito species such as *Anopheles gambiae* and *A. arabiensis* in Africa and *A. culicifacies* in India are commonly found in the turbid water created by puddling (Mather and Trinh 1984).

Fallow fields flooded before plowing and rice nurseries produce high densities of *A. culicifacies* in India. Subsequent cultural operations such as plowing reduce densities, and the species generally does not occur after the rice plants reach a height of 30 cm (Russel et al 1942).

7.8.1.3. *Pesticides.* **Synthetic pesticides.** Most agricultural insecticides are not specific; they are toxic to rice pests as well as to some vectors and their predators/competitors. Insecticides, however, are primarily applied to con-

trol rice pests and their effects on vectors are mostly unplanned (see sect. 6.3.4.5). Similarly, the control of snail vectors can be achieved with molluscicides, but those are used for controlling rice pests (see sect. 7.8.2) rather than snail vectors, for which application would be uneconomical.

**Botanical pesticides.** Because of the economic limitations of synthetic pesticides for vector control, research emphasis has been placed on the potential of cheap, botanical pesticides. Many studies have shown the insecticidal properties of oil from the seed kernel of the neem tree (Saxena 1989). Another oil derived from the custard apple (*Annona* spp.) also acts as an antifeedant to several rice pests. These products have been employed in a crude form as mosquito repellents for many years in some Asian countries. More recently, neem oil has been shown capable of affecting and even killing mosquito larvae (Mather and Trinh 1984, Zebitz 1986, Saxena 1989). A possible problem with plant material used directly as a pesticide is the variability of the properties that depend upon the growth conditions and the time and methods of harvest and conservation. Other botanical pesticides have been reported to economically control molluscs. Nuts, berries, or shells of *Phytolacca dodecandra*, *Croton tiglium*, and *Anacardium occidentale* reportedly controlled some snail populations (McCullough et al 1980, Webbe and Lambert 1983). Hostettman (1984) reported that approximately 50 molluscicidal compounds have so far been isolated from plants. They include saponins, terpenoids, flavonoids, naphthoquinones, and tannins. Gaddal (1988) pointed out that while in the 1970s plant molluscicides were thought to be the answer to snail control and that rice-growing countries would be able to produce their own, enthusiasm in Africa has waned for the two main candidate molluscicides, *Phytolacca dodecandra* and *Damsissa*.

The wide range of plants exhibiting insecticidal or molluscicidal properties offers an alternative for vector control that is underexploited.

7.8.1.4. *Biological control.* Biological control of vectors has two major approaches: 1) conserving natural predators and maintaining species diversity, and 2) introducing new predators (fish or insects), competitors, and parasites (microorganisms or nematodes).

**Conserving natural predators.** Although many vectors can exist in traditional ricefields, the diversity of animal populations and predator pressure is believed to limit productivity of any one vector. In contrast, strict rice monoculture will have a less diverse fauna, and, without control measures, the productivity of a few key vectors may be high. The insecticides needed to manage that situation may create secondary problems (Bradley 1988). Major vector predators in ricefields are fish that feed on mosquito larvae and snails, and aquatic insects and their larvae that feed on mosquito larvae. Insect predators of mosquito larvae include back swimmers, Gerrids, etc. (Hemiptera, Notonectidae), dragonfly and damselfly nymphs (Odonata), and predacious water beetle adults and larvae (Coleoptera, Dytiscidae) (Service 1977). In Kenya, about 30 species of predators of *Anopheles gambiae* occur in ricefields, the most

important belonging to Amphibia, Odonata, and Coleoptera (Service 1977). In Japan, about 30 species were confirmed to feed on mosquito larvae in the laboratory (Sugiyama et al 1985, Watanabe et al 1968). The most important were insects belonging to Odonata (dragonfly and damselfly, nymphs), Notonectidae (back swimmer, nymphs and adults), Dytiscidae (predacious water beetle, adults and larvae), and three species of fish: *Orizias latipes*, *Carassius gibelie*, and *Misgurnus fossilis* (Mogi 1988). Predator fauna vary considerably according to rice cultivars grown, height of the rice plant, and water management (Mather and Trinh 1984).

Most of the information on conservation of natural predators refers to mosquito predators. Some are important in reducing the number of mosquitoes, at least under certain circumstances (Mogi 1978, Wada 1974). For example, the number of adults produced from 3,300 1st-instar larvae of *Culex tritaeniorhynchus* placed in each of 3 experimental plots ranged from 0 to 144. The number of Odonata and Notonectidae nymphs and adults was larger in the plot from which fewer adult mosquitoes emerged, indicating their importance as predators (Mogi et al 1980a,b).

Survival of mosquito larvae in ricefields from the 1st-instar through the pupal stage varies from 2 to 5% (Mogi et al 1980a,b, Northup and Washino 1983, Service 1977). Much of the natural mortality is attributed to fish and insect predators (Miura et al 1978, Mogi 1978, Service 1975, Washino 1981), but spiders are also important in reducing the number of adult mosquitoes (Wada 1988). It is, however, difficult to determine which predator or groups of predators are the most important, and that may vary considerably from one geographic area to another (Dame et al 1988).

Although predators of mosquito larvae may well be causing 90% or more mortality of the immatures, there may still be large numbers of mosquitoes emerging and constituting a nuisance or disease hazard. If these natural predators are destroyed, then the numbers of emerging mosquitoes are likely to be even greater. Therefore, even if it is not envisioned that invertebrate predators can be exploited as biological control agents, cultural practices favoring their existence should be encouraged (Mather and Trinh 1984).

**Fish introduction.** Food fish have been used, purposely or not, for vector control in many Asian countries. In China, raising grass carp and other fish in ricefields led to an 80-90% reduction in the density of mosquitoes and their larvae in rice-growing areas in Hunan Province. Grass carp ingested about 400 mosquito larvae/d (Anon. 1984, cited by Petr 1987). The exotic Nile tilapia, now widely used in tropical Southeast Asia, is also an efficient larvivore (Petr 1987).

According to Dame et al (1988), *Gambusia affinis* or mosquito fish is still the only demonstrated biological control agent presently used in the USA for ricefield mosquito control. The fish is introduced into ricefields annually, shortly after initial flooding. Once established, several broods may be produced during the summer months. Approximately 750 fish/ha give effective

control (Hoy et al 1971). Stocking fields 15-25 d after rice seeding appears to give the best fish population growth and mosquito control (Farley and Younce 1977). Haas and Pal (1984) reported that *G. affinis* effectively controls mosquitoes because it 1) easily penetrates shallow, weedy areas, 2) is primarily carnivorous but becomes omnivorous when food is scarce, 3) has a dorsal mouth and frequents the surface, 4) bears live young, and 5) tolerates salinity, high temperature, and moderate organic pollution. Several authors, however, question the use of *G. affinis* in ricefields. It has been effective against *Culex tarsalis* in California ricefields, but its effectiveness against *Anopheles* spp. is questionable (Hoy et al 1971). Farley and Younce (1977) considered it a rather poor control agent as it preys to a large extent on nontarget organisms, such as crustaceans and chironomids. Because it preys on eggs of other fish, its introduction might reduce indigenous fish species (Schaefer and Meisch 1988). Also, the fish appeared to prefer the deeper, open portions of water in the ricefields, while mosquito larvae were most abundant in the shallow, protected areas. Thus, the predator and prey were isolated from each other ecologically (Reed and Bryant 1972). *Gambusia* invaded parts of Japan and drove a native fish, occupying the same ecological niche, almost completely out of the ricefields. Therefore, it added little to the role of natural enemies in mosquito control (Wada 1988).

Experience in the efficacy of exotic larvivorous fish varies. Mather and Trinh (1984) said that there is little real evidence to show that introducing fish into habitats already colonized by a rich fauna of natural predators has led to any worthwhile reduction in disease transmission. Indigenous larvivorous fish have already been shown to be highly effective in mosquito control. Such fish are well adapted to local conditions and are readily available for mass rearing and dissemination. This underscores the importance of careful studies before introducing any exotic natural enemies. The most promising concept is to stock fields with larvivorous fish that can later be harvested for food. Research is needed to identify indigenous species more suited to the ricefields of individual countries.

Black carp *Mylopharyngodon piceus* and common carp can be used for controlling snails (Mochida 1988).

**Insect predators.** Probably the least attention to date has been given to insect predators. Numerous aquatic predator species occur in most ricefields, but their potential for mosquito control for most parts of the world is unknown. Their taxonomy and ecology need to be studied before their possible use in integrated control can be assessed or ways of multiplying them can be evaluated (Schaefer and Meisch 1988).

**Animal competitors.** Use of competitors is a strategy that seems to have been restricted to snail vectors. The use of competitor snails, *Marisa cornuarietis* and *Thiara*, to supplant schistosome vector snails has been successful in ponds and canals (Gaddal 1988).

*M. cornuarietis*, a large ampullarid, was used successfully in large ponds in Puerto Rico as a competitive feeder and incidental predator on *Biomphalaria glabrata*. It replaced *B. pfeifferi* in an aquatic habitat in Tanzania and in the canals of the Gezira scheme in Sudan (Gaddal 1988). *M. cornuarietis* was reported to destroy newly transplanted rice (Gaddal 1988), but Bergquist and Chen (1988) consider the danger to rice seedlings to have been exaggerated. *Marisa* is not particularly tolerant of its habitat drying out over prolonged periods (Nguma et al 1982). Mather and Trinh (1984) neither consider *Marisa* use a proven method to control snail vectors in rice irrigation systems nor do they recommend it.

*Thiara* is reported to have caused the decline in *B. glabrata* populations in certain West Indies islands. Another snail with the potential to replace the schistosome vectors in some African field situations is *Helisoma duryi*, which appears to combine direct competition and growth-inhibiting factors to replace snails in freshwater habitats (Gaddal 1988).

The reduction of the snail population by introducing sterile males has not shown promise (Garcia 1988).

**Azolla for mosquito control.** The potential negative effect on mosquito breeding of water surface coverage by *Azolla* was suggested in China as early as 1934 by Li and Wu. Laboratory studies of the effect of *Azolla* on mosquito breeding show that complete coverage of the water surface by *Azolla* is needed to inhibit oviposition of *Culex* spp. (Lu Baolin 1988). On the other hand, *Azolla* is a good source of food for snails. Therefore, it is highly unlikely that *Azolla* will be used as a method for mosquito control only. Rather mosquito control is an additional benefit in areas where *Azolla* is used as green manure.

**Microbial agents.** Microbial agents with the potential to control mosquitoes include viruses, bacteria, and fungi (Lacey and Undeen 1986).

*Bacillus thuringiensis* serotype H-14 (sometimes designated H-14 or Bti) has been the most studied agent. It can provide selective control of mosquito larvae, causing relatively little harm to most of the predators of vectors and agricultural pests (Way 1987). The application of Bti to ricefields in Louisiana, USA, is often accomplished by a drip technique, which is calibrated to match the flow of irrigation water into the fields. Thus, the bacterial spores containing the toxin are present when the vector larvae of *Psorophora* hatch shortly after flooding. The level of control is usually high except in areas where the hydrological characteristics of the field restrict water movement. Because the vector hatches in response to flooding, the brood can be controlled by a single treatment of about 1.2 liters/ha (Mc Laughlin and Vidrine 1984).

Other microbial agents with a potential to control malaria vectors include *Bacillus sphaericus*; fungi such as *Culicinomyces clavisporus*, *Lagenidium giganteum*, *Tolypocladium cylindrosporum*, and *Coelomonzyces* spp.; and viruses such as baculoviruses (Cowper 1988).

The high levels of mortality produced by several virulent strains of *B. sphaericus* in several culicine and anopheline larvae—without undue effect

on nontarget organisms—and the persistence of its larvicidal activity make it a viable candidate for testing in ricefields (Dame et al 1988).

The genera of fungi *Coelomomyces*, *Lagenidium*, and *Culicinomyces* have the ability to recycle and are efficacious in the field. *Lagenidium* and *Culicinomyces* also offer the advantage of being culturable on artificial media. In the USA, *Lagenidium giganteum* has been successfully field-tested in ricefields in California (Kerwin and Washino 1986a), and, to a lesser extent, in Arkansas (Dame et al 1988)

**Nematodes.** Some nematodes have the potential to control malaria vectors, especially *Romanomermis culicivorax*. Because of its demonstrated recycling and apparent resistance to desiccation during winter, it has been investigated in California ricefields (Kerwin and Washino 1986b, Westerdahl et al 1982). Minimal parasite dispersal from the point of application in ricefields, as well as the labor required for mass rearing in vivo, renders this approach impractical now (Dame et al 1988, Schaefer and Meisch 1988).

7.8.1.5. *Conclusions.* It is highly improbable that rice farmers will adopt measures designed to control vectors only. To be attractive, vector control practices should be part of cultural practices that show tangible benefits in increased yield or reduced input.

In many areas, farmers use more pesticides than are required for controlling rice pests. Although natural biological control is not expected to entirely prevent pest damage, neither is the use of pesticides. There is sufficient evidence that destruction of natural populations of predators through overuse of pesticides can lead to the development of undesirable vector species and pesticide resistance.

With regard to the potential of food-fish for controlling vectors, it is important to investigate rice cultural practices, including methods of pesticide use, that are compatible with fish culture.

More research on conservation of environmental conditions that sustain natural enemies of vectors is also needed.

Among the possible methods of water management for vector control in ricefields, the alternate wetting and drying method seems more promising than the others because of its potential for water savings. The method, however, will also affect natural predators and competitors of the vectors. There is a need to establish the relation to yield of alternate wetting and drying for various soil types and environmental conditions. The cost of water control infrastructure for intermittent irrigation may not be very high, but for sustained success it will require a level of farmer organization and institutional support not currently available in most tropical rice irrigation systems.

The potential of biological control has wide appeal to field biologists, but no method has yet been sufficiently evaluated to be recommended.

Among possible methods, the most promising for small farmholders is to stock ricefields with larvivorous fish that could be harvested later for food. Research is needed to identify fish species more suitable to the ricefields than

those that now exist in individual country situations. In addition, there is a need to develop pest management technologies, including establishing maximum doses, which are compatible with fish culture, when pesticides are deemed essential.

It seems that mosquito control by *Bacillus thuringiensis* has had some success in the USA. The efficacy and economics of the technique should be tested under tropical conditions.

### 7.8.2. Control of soil and water fauna detrimental to rice

Large aquatic snails are probably the floodwater organisms most often detrimental to rice. They are also among the most difficult to control (Acosta and Pullin 1991). Three groups of control methods available to farmers include cultural practices, biological control, and chemical control. Descriptions of the individual methods and their limitations follow.

Cultural practices:

- Retransplanting missing hills. Limitations: added cost, results in nonuniform maturity that can reduce grain quality.
- Lowering the floodwater to < 1 cm for few weeks after transplanting. Limitations: promotes weed growth, method not applicable in waterlogged areas or when there is heavy rainfall.
- Hand picking of animals and egg masses. Limitation: added cost, but collected snails can be used as food or feed for ducks. Hand picking can be facilitated by 1) constructing small canals, where snails will concentrate when field are drained, and 2) placing sticks in the field to attract snails for egg deposition.
- Providing attractants or alternative feeds to rice seedlings. Leaves of papaya *Carica papaya*, kankong *Ipomoea aquatica*, sweet potato *Ipomoea batatas*, cassava, or taro divert snails from rice seedlings and facilitate their collection. Newspapers and sacks were found to be alternative attractants.
- Placing metal screens at irrigation inlets to prevent entry of snails into ricefields from irrigation canals.

Biological control:

- Introducing snail-eating fish such as black carp and common carp into the floodwater. Limitation: the method has not proved very successful (Acosta and Pullin 1991).
- Introducing ducks to eat snails. Limitation: ducks are an intermediate host for a trematode whose free-living larvae pierce human skin with their stylets, causing itching to farm workers exposed to them in the ricefields.

Chemical control:

The major molluscicides used to control the golden snail are organo-tin compounds (Aquatina, Brestan, and Telustan) and Niclosamide (Bayluscide). Molluscicide use is limited because 1) most farmers cannot afford them, 2)

they are highly toxic to fish, frogs, and other beneficial organisms, and 3) some adversely affect humans working in the fields. Because molluscicide must be applied before transplanting to be effective, farmers are exposed to it during transplanting. Farm workers have suffered damage to their skin and nails from working in fields treated with molluscicides. Tin-based molluscicides are especially effective in eliminating snails, but they are very toxic to other animals and the true cost of their use has not yet been estimated.

The only molluscicides that would fit well into the cropping system would be those that are nonpersistent, have low toxicity to humans and fish, could be applied after transplanting, and are low-cost. There are currently none available that combine all of those properties (Acosta and Pullin 1991).

The methods listed above work at different levels, and combinations of methods are more efficient than a single method used alone. In the Philippines, about half of the farmers rely only on molluscicides, while the other half use molluscicides in combination with any of the following methods: application of increased doses of insecticides, hand picking, construction of trenches, and intermittent irrigation.

Fresh-water crabs (*Paratelphusa hydromus* and *P. spinigera*), which cut the stems of deepwater rice and decrease yield, can be controlled with boxlike traps using snail meat as bait. Moreover, the crabs are edible and provide an additional source of food (Mukhopadhyay et al 1992).



## 8 Summary and conclusion

The flooded ricefield is a temporary aquatic environment whose major characteristics are determined by flooding, the presence of rice plants, and agricultural practices. Flooding creates anaerobic conditions a few millimeters beneath the soil surface and leads to the differentiation of five major environments distinguished by their physical, chemical, and trophic properties: floodwater, surface-oxidized soil, reduced soil, rice plants (submerged parts and rhizosphere), and subsoil.

The ricefield floodwater is a photic, aerobic environment where communities of chemosynthetic and photosynthetic producers (bacteria, algae, and aquatic plants), invertebrate and vertebrate primary consumers (grazers), and secondary consumers (carnivorous insects and fish) provide organic matter (OM) to the soil and recycle nutrients. The physical and chemical properties of the floodwater sometimes vary markedly both during the day and the crop cycle. Daily variations of 10 °C, 2 pH units, and 15 ppm dissolved O<sub>2</sub> are not unusual, especially at the beginning of the crop under a weak canopy. Marked variations in dissolved chemicals result from fertilizer application.

### 8.1. Primary producers in floodwater

The photosynthetic aquatic biomass (PAB) that develops in ricefield floodwater is composed of cyanobacteria; planktonic, filamentous, and macrophytic algae; and vascular macrophytes. Its average daily primary production ranges between 0.2 and 2.0 g C/m<sup>2</sup> per d. That corresponds to 10-15% of rice primary production and is similar to that in eutrophic lakes. Standing crops usually range from 100 to 500 kg dry weight (dw)/ha. The average composition of aquatic macrophytes is 8% dry matter, 2-3% N, 0.2-0.3% P, and 2-3% K. Planktonic algae have higher N contents, averaging 5%. Algae aquatic macrophytes are characterized by both high water (90-99%) and ash (20-70%) contents. The N content of spontaneously growing PAB in planted fields rarely exceeds 10-20 kg/ha but might reach 30-40 kg/ha in flooded fallow fields.

Light availability influences PAB activity and size. Light intensity influences the nature of dominant components. To a lesser extent, temperature also affects both the production and the composition of PAB. Soil pH and agrochemicals (nature, quantity, and application technique) have a strong in-

fluence on the algal flora composition. N fertilizer broadcasting, as a general rule, favors eukaryotic algae and inhibits N<sub>2</sub>-fixing cyanobacteria. Phosphorus application favors the growth of N<sub>2</sub>-fixing phototrophs if no other factor is limiting. Herbicides and algicides directly affect PAB. Insecticides mostly affect it indirectly by reducing populations of primary consumers.

The major aspects of the N cycle involving PAB follow.

- Photodependent N<sub>2</sub> fixation, which ranges from 0 to 30 kg N/ha per crop, depending on cyanobacteria growth. It can be higher if *Azolla* is grown.
- Immobilization of N from floodwater and surface soil, which may correspond to 5–40% of N fertilizer applied.
- N recycling in relation to spontaneous death or grazing of primary producers, their decomposition resulting in N accumulation at the soil surface, and its translocation to deeper soil by soil fauna.
- Contribution to the replenishment of the soil microbial biomass.
- Provision of N to the rice plant corresponding to a recovery, which varies from 10 to 40%, depending on the nature of the material, and the method of application.
- Induction of N losses by NH<sub>3</sub> volatilization through increasing floodwater pH. Losses may be as high as 30–60% of fertilizer applied.

## 8.2. Fauna

Many species of large aquatic vertebrates (fish, frogs) and invertebrates (shrimp, crayfish, snails) are found in ricefield floodwater. They have been traditionally collected as food. The dominant micro- and meso-invertebrates are ostracods, copepods, cladocerans, rotifers, insect larvae, aquatic insects, molluscs, oligochaetes, and nematodes. They have agricultural and environmental impacts as nutrient recyclers, rice pests, pest predators, and vectors of human and animal diseases.

Ostracods, mosquito, and chironomid larvae are major grazers that can develop population densities up to  $4 \times 10^4/\text{m}^2$ . Aquatic snails can develop populations up to  $10/\text{m}^2$  for large species and  $1000/\text{m}^2$  for small species. They feed on algae and decomposing OM. Algae that form mucilaginous colonies, or large filaments, or produce toxins are less susceptible to grazing than other strains. Grazers can consume as much as 50–100 kg fresh weight (fw) algae/ha per day.

Translocation of primary production and its breakdown products to the deeper soil is expedited by aquatic oligochaetes, mostly tubificids, which develop populations up to  $4 \times 10^4/\text{m}^2$  (630 kg fw/ha). By moving back and forth between the reduced soil and the floodwater, they 1) increase exchanges between both environments, 2) stimulate soil OM decomposition, 3) concentrate large soil particles and weed seeds in the lower soil, which reduces weed

incidence, and 4) increase the activity and the biomass of bacteria and aquatic flora and fauna in floodwater.

Invertebrate predators, which contribute to nutrient cycling and the control of pest outbreaks, include species of Odonata, Coleoptera, and Notonectidae. Tertiary consumers (carnivorous insects and fish) also recycle nutrients or cause an exportation of nutrients when they are collected for food.

Aquatic vertebrates and invertebrates reported as rice pests include some fish, tadpole shrimp, crabs, snails, and microcrustaceans feeding on rice seedling and their roots. Snails that feed on rice seedlings are probably the most detrimental and are considered major pests in several countries.

An important aspect of ricefield fauna is the occurrence of snails and mosquitoes that are vectors of human and animal diseases such as malaria, schistosomiasis, and Japanese encephalitis.

### 8.3. Effects of crop intensification on floodwater ecology

Although modern technologies, which use fertilizer-responsive rice varieties, fertilizers, pesticides, and optimum management practices, have increased yields tremendously, they have profoundly modified traditional rice-growing environments. It is generally recognized that disturbances of the ecosystem by mechanization and agrochemicals and the disappearance of permanent reservoirs of organisms in the vicinity of the fields have decreased species diversity and the number of edible species traditionally harvested from the ricefield. However, quantitative knowledge of changes in species diversity in ricefields is extremely scarce. Available data indicate but do not conclusively demonstrate a decrease in biodiversity after crop intensification.

With regard to floodwater ecology, the greatest pressure exerted on the faunal and floral communities of ricefields is agrochemical use. Both fertilizers and pesticides have significant impacts on population composition and dynamics. Their application frequently leads to explosive growth of single species of algae or invertebrates that might directly or indirectly have detrimental effects.

Broadcasting N fertilizer into the floodwater, besides increasing rice yield, induces the upsurge of blooms of eukaryotic algae, which 1) causes marked increase in water pH and favors N losses by  $\text{NH}_3$  volatilization, 2) directly and indirectly inhibits biological  $\text{N}_2$  fixation by cyanobacteria, and 3) causes the proliferation of grazers, including detrimental mosquito larvae. These negative impacts can be avoided by deep placement of N fertilizer.

A wide range of pesticides is used in wetland ricefields to control rice pests and weeds. They also affect nontarget cyanobacteria, microalgae, and floodwater invertebrates. When interpreting the results of laboratory and field studies on the impact of pesticides on nontarget ricefield organisms, special attention must be paid to methodological aspects because of several possible biases. Information from *in vitro* studies can only partly be extrapolated to

the field where conditions differ markedly and pesticide degradation is usually much faster. Also, many studies have used pesticide concentrations far higher than those resulting from field application at the recommended level. Experimental designs testing impacts of pesticides in the absence of fertilizer are also of limited value because such a situation is uncommon in farmers' fields and might exaggerate pesticide effects.

In field studies, two major effects of pesticides on ricefield algae have been reported: 1) a general selective toxicity, which favors cyanobacteria because they can often tolerate high levels of pesticides, and 2) a growth-promoting effect of insecticides on algae due to the decrease of grazer populations. Ricefield algae can significantly contribute to the bioconcentration of pesticides. That aspect is important when considering the ricefield as an environment for aquaculture.

Insecticides are the most active pesticides on floodwater invertebrates. Their major reported effect on zooplankton is decreasing species diversity and causing blooming of individual species, especially ostracods, tubificids, and mosquito larvae, while populations of predators such as odonate larvae decrease. There are also reports indicating a limited effect of pesticides on zooplankton. Recent field studies indicate that zooplankton are more affected by N fertilizer than by pesticides applied at the recommended dose. Available data indicate that aquatic oligochaetes are sensitive to pesticides, but they are insufficient to permit drawing definite conclusions. Snails are usually not affected by conventional rice pesticides other than molluscicides, but their populations may increase because of reduced competition for energy sources. Insecticides are reported to have three major effects on vectors: 1) they temporarily decrease their incidence, 2) they cause resurgence of resistant strains, and 3) they have adverse effects on the natural predators and competitors of vectors.

Possible impacts of crop intensification on long-term soil fertility are far from being fully assessed. Preliminary experiments show that under intensive rice cultivation, management practices that deprive primary producers of floodwater (combination of pesticide use, intense weeding, and a dense rice canopy) may significantly reduce soil microbial biomass and thus long-term soil fertility. Both N fertilizer and pesticides seem to rather increase zooplankton activity. Not only might pesticides decrease populations of aquatic oligochaetes, but they might also decrease the translocation of nutrients from the detritus layer to the deeper soil layer, thus reducing soil fertility. Currently no data are available to substantiate these hypotheses. The knowledge on impacts of pesticides on wetland soils is also too fragmentary to draw conclusions other than general trends. Impacts of pesticides on the soil-floodwater ecosystem can be significant without being detrimental and should be considered in the context of ecosystem equilibrium and not in isolation.

## 8.4. Agronomic management of the photosynthetic aquatic biomass

### 8.4.1. Control of detrimental algae and aquatic macrophytes

Microalgae are usually not considered detrimental in transplanted rice except by favoring N losses by  $\text{NH}_3$  volatilization. In direct seeded rice, they are responsible for the so-called *slime* or *scum*, which can be detrimental at germination because of competition for light and mechanical effects on seedlings. Such blooms most often have been controlled by Cu-based algicides. They can also be controlled by draining the field, but farmers are often reluctant to do so because of water wastage. Submerged and floating macrophytic weeds can sometimes develop very large biomasses and cause significant yield losses when growing early in the crop cycle. Mechanical or manual control is not feasible because it is costly and injurious to the crop. Chemical weed control has increased yield by 20–50%, but such situations are highly localized.

Ammonia volatilization results from a marked increase in floodwater pH due to algal activity when  $\text{NH}_3$  concentration in floodwater is high. Therefore practices that decrease algal growth and prevent the buildup of  $\text{NH}_3$  in the floodwater reduce  $\text{NH}_3$  volatilization. Algicides tested to improve fertilizer efficiency decreased water pH for a few days, but N saving was low (5–10 kg N/ha) and yield increase was undetectable. The buildup of  $\text{NH}_3$  in the floodwater following fertilizer application could also be prevented by inhibiting urease, which hydrolyzes urea to ammonium bicarbonate. However, recent studies concluded that an ideal inhibitor could increase yield by a maximum of 64%. More data are needed for definite conclusions, but deep placement of N fertilizers, which forestalls the rapid growth of non- $\text{N}_2$ -fixing algae and prevents high concentration of  $\text{NH}_3$  in floodwater, seems a more efficient method to increase fertilizer efficiency.

### 8.4.2. Use of aquatic macrophytes as organic fertilizer

Standing crops of algae and aquatic weeds often attain 1–5 t dw/ha in irrigation canals and water tanks. Higher values (30 t dw/ha) have been reported in water receiving farm or factory effluents. Their dw composition is very similar to that of many green manures except for K in macrophytes and N in planktonic algae, which are higher. Using aquatic weeds from water bodies as a manure permits the reclamation of the water body or the removal of wastewater effluents and the fertilization of a crop with an organic manure frequently rich in N, P, and K. Available data show that aquatic plants have been used as a source of OM and nutrients mainly in dryland soils and that their potentials on wetland soils are poorly documented, except in China. Positive effects on grain yield have been reported, but detrimental effects such as weed dispersal and concentration of pesticides and heavy metals are possible. Because of the bulkiness of aquatic weeds and the occasional need for composting before incorporation in the field, their use will be strongly influ-

enced by agro-economic conditions, the facility of harvest, and the distance between the field and the places of harvesting and composting.

#### 8.4.3. Use of *Azolla* as green manure

The use of *Azolla* as a green manure takes advantage of the symbiosis between a fern of the *Azolla* genus and a N<sub>2</sub>-fixing cyanobacterium. The N potential is similar to that of legumes. *Azolla* is widely distributed throughout the world but needs to be inoculated and cultivated to develop significant biomass in ricefields. It is easier to incorporate than other green manures and grows well with rice in flooded conditions. Field trials conducted for 4 yr at 37 sites in 10 countries showed that 1) incorporating one crop of *Azolla* grown before or after transplanting is equivalent to application of 30 kg N/ha, and 2) incorporating two *Azolla* crops grown before and after transplanting is equivalent to split application of 60 kg N/ha. Besides being a N source, *Azolla* concentrates K from water and is a source of K for rice when incorporated. It also decreases weed incidence and water evaporation and improves soil structure.

Despite this potential, the area devoted to *Azolla* use greatly decreased during the 1980s in Vietnam and China, where it had been traditionally used, neither has it spread in other countries, which had studied the feasibility of its adoption by rice farmers. World use is now only a fraction of the estimated 2 million ha of rice that were fertilized with *Azolla* in China and Vietnam in the late 1970s.

Environmental, technological, and economic factors limit *Azolla* use. Because *Azolla* is propagated vegetatively, inoculum must be maintained in nurseries year-round and multiplied for distribution before field growth. The optimum temperature for most species (20–30 °C) is below the average temperature in the tropics. Cool weather is a key to successful *Azolla* cultivation in Vietnam and China. Insect pests and diseases limit *Azolla* growth in the humid tropics. Phosphorus application is required for growing *Azolla* in most soils. Because *Azolla* technology is labor-intensive, it also has economic limitations. In areas of the Philippines where conditions for *Azolla* growth were not favored by an exceptionally high level of available P, *Azolla* use was uneconomical.

Problems in inoculum conservation, multiplication, and transport could be solved if *Azolla* could be propagated from spores. Temperature limitations and P requirements can be reduced by selecting cold- or heat-resistant strains with low P requirements and by limiting the applications of P fertilizer and pesticides to inoculum production. Recent progress in strain hybridization and recombination opens new possibilities to overcome environmental and nutritional limitations. Socioeconomic limitations are important and are probably increasing, as shown by the setback in China and Vietnam. But economic calculations should also consider the long-term benefits of *Azolla* on soil fertility.

Recent studies have shown that *Azolla* can also be a weed suppressor, a K source through its ability to concentrate the element, an animal feed, a primary producer in rice-fish-*Azolla* culture, and that it can also decrease N losses by reducing NH<sub>3</sub> volatilization. The major limiting factors to its use as a green manure (especially economic feasibility) and its potential as a multipurpose crop will decide the extent of its future use.

#### **8.4.4. Use of free-living cyanobacteria as biofertilizer**

Biomass estimates, acetylene-reducing activity measurements, and inoculation experiments indicate that as an additional N source for rice, cyanobacteria have a potential of about 20-30 kg N/ha, which may translate to a yield increase of 300-450 kg/ha. Cyanobacteria may also increase yields by increasing P availability and soil properties, and possibly by producing growth-promoting substances—but currently, no report shows their isolation or characterization. In most inoculation experiments, only yield was measured. No data on environmental conditions, cyanobacteria establishment and biomass, or N<sub>2</sub>-fixing activity in successful inoculation experiments are available. Therefore, reasons for the yield increase are still unclear, especially in cases when a beneficial effect was observed with high levels of N fertilizer, which reportedly inhibit cyanobacteria growth.

Because of a very limited knowledge of factors that allow inoculated cyanobacteria to establish and bloom in ricefields, cyanobacteria inoculation is conducted on a trial-and-error basis, and its effects seem to be erratic and frequently small. That may explain why algal inoculation, known for at least 30 yr, is practiced on only a limited hectarage in a few Indian states and possibly in Myanmar.

Most agronomic studies on cyanobacteria have emphasized inoculation with laboratory-selected strains. That arose from the earlier belief that N<sub>2</sub>-fixing strains were not present in many ricefields. Recent results show that 1) they are ubiquitous in rice soils, 2) foreign strains are rarely established, and 3) attention should be paid to agricultural practices that will alleviate limiting factors in fields where cyanobacteria are present but do not bloom. Practices to enhance cyanobacteria growth include P application, grazer control, deep placement of N fertilizer, and liming. Economic feasibility, however, has to be considered. In particular, efficiency for applied P is low (2.3 g N/g P) and chemical pesticides are too expensive to control grazers considering the expected benefit. Grazer control by floodwater management and botanical pesticides might be economically feasible. Inoculation with indigenous strains might be useful in soils where initial density of cyanobacteria has been decreased by a long dry fallow or an upland crop. A P-rich inoculum applied at the soil surface has an initial advantage over the indigenous propagules, which are usually P-deficient and mixed with the soil.

Cyanobacteria are unlikely to be an exclusive N source for producing high yields. Their potential is yet to be expressed in integrated nutrient man-

agement. Combining N fertilizer deep placement with other practices that favor cyanobacteria growth is a possible method. Another would be to delay fertilizer application to allow an early N<sub>2</sub>-fixing bloom to grow, but the resulting effects on N losses from fertilizer applied in an algal-rich water have to be tested. In-depth agroecological research is still required before cyanobacteria technology can be substantially improved.

## 8.5. Agronomic management of floodwater fauna

### 8.5.1. Rice-fish culture

The practice of collecting naturally occurring fish for food from ricefields is probably as old as rice cultivation itself. Capture systems still occupy a greater area than culture systems and are important in many rice-growing areas of Asia. But crop intensification has caused a rapid decrease in fish catches, and farmers may have to change to culture systems to maintain their income from fish.

There is a wide array of rice-fish systems resulting from the combinations of

- field design: trench(es) within the field, pond adjacent to the field, and deepwater ricefield;
- types of growing period: concurrent with rice and rotational, where fish are grown as a single annual crop after rice or as an intermediate crop between two annual rice crops;
- fish species: grown singly or in association (prawns, shrimp, and crayfish are also grown); and
- types of production: growout fish or fingerlings.

Fish yield depends on the species stocked, the culture period, the fertility of the soil and water, and the degree of supplemental feeding. Reported yields range from 10 to 600 kg/ha. Besides fish production, rice-fish culture may have other benefits, among them: 1) control of rice pests—insects, weeds, and snails—and human disease vectors, 2) improvement of physicochemical properties of soil, 3) possible reduction of N losses due to NH<sub>3</sub> volatilization, 4) increase in nutrient cycling and availability, and 5) increase in rice yield and N uptake. In particular, published data indicate an average 15% increase in rice yields calculated on the basis of the area planted to rice. Experimental evidence to support these reported positive agroecological interactions, however, is rather meager.

Major technical constraints to fish stocking in ricefields are 1) additional work and monetary investments, 2) availability of fry or fingerlings, 3) impacts on rice cultural practices, and 4) detrimental effects of pesticides on fish. In particular, concurrent culture may be currently restricted because of indiscriminate use of insecticides. There is evidence, however, that pesticides do not necessarily restrict the adoption of rice-fish culture. Research on rice-fish culture is also directed toward the supportive role for integrated pest

management (IPM), which could act as a vanguard for rice-fish culture expansion. Rice-fish culture creates added costs for the farmer in terms of greater input and management requirements, but economic analyses often show a high return.

Rice-fish systems presently occupy only a very small percentage of the potential area. In countries where the capture system has been traditionally practiced, there is a high potential to upgrade the rice-fish pattern toward culture systems. In irrigated and deepwater environments, where culturing fish is not traditional, interest in rice-fish farming is growing because it is a promising alternative to rice monocropping by diversifying production. Moreover, it offers new opportunities for farmers to supplement their income, ensures a better food security, enforces a sound use of agrochemicals, and favors IPM and biodiversity conservation.

### **8.5.2. Control of detrimental fauna**

The control of vectors of human and animal diseases that develop in ricefields can be achieved, at least partly, by water management, agricultural practices such as synchronous planting and harvesting, pesticide application (including botanicals), and biological control. It is highly improbable, however, that rice farmers will adopt measures designed to control vectors only. To be attractive, vector control should be part of cultural practices that increase yield or reduce inputs. Among the possible methods of water management, the alternate wetting and drying method seems promising because of its potential for water saving. The wide range of plants exhibiting insecticidal or molluscicidal properties offers an alternative for vector control, which is underexploited. The potential of biological control has wide appeal to field biologists, but no method has yet been sufficiently evaluated to warrant its recommendation. Use of competitors was reported to be successful for snail vectors in a few irrigation schemes. The most promising method for small farmholders is obviously to stock ricefields with larvivorous fish that could later be harvested for food.

Large aquatic snails are probably the floodwater organisms most often detrimental to rice. They are also among the most difficult to control. Numerous management and biological control methods have been tested, but their adoption is limited and molluscicide use is increasingly practiced by farmers in concerned areas.

## **8.6. Research needs**

From the point of view of yield sustainability, traditional wetland rice has been extremely successful. Moderate but stable yields have been maintained for hundreds of years without deterioration of the environment. Flooding maintains the biological and chemical fertility of the ricefield ecosystem through the diversification of microbial environments and the establishment of an aquatic community. The sustainability of low-input, traditional ricefields

is largely due to the activity of PAB of a few hundred kg dw/ha whose  $N_2$ -fixing activity and turnover is preponderant in recycling nutrients and replenishing available N in the ecosystem.

Improved rice varieties and technologies have been successful in increasing yields, but have markedly modified the ricefield ecosystem. Earlier concerns were how to increase yield, optimize agrochemical use, identify and use alternative cheap sources of fertilizer, and, to a lesser extent, preserve the ability of the ricefield to produce additional sources of food. New concerns deal with sustainability of the rice-growing environment to maintain high yields and the possible long-term effects of crop intensification and factors associated with it—especially agrochemical use—on soil fertility, the environment, and human health.

A number of techniques have been studied or proposed, aiming either at using the agronomic potential offered by the ricefield floodwater ecosystem and its communities, or alleviating some of their detrimental effects (Table 8.1). However, none has been widely adopted. The two most successful practices (*Azolla* and rice-fish culture) are used at best in about 1% of the world rice-growing area (148 million ha).

To ensure the sustainability of rice-growing environments, major progress must be made in our understanding of ricefield floodwater ecology and in the development of agroecological methods to manage it.

As N is usually the limiting factor to high yields in ricefields, earlier research on photosynthetic aquatic organisms focused on the use of photodependent  $N_2$ -fixing organisms as an alternative or supplementary N source for rice. Biofertilizer technologies currently used by rice farmers (green manuring with legumes or *Azolla*) are labor-intensive and most often have been used under socioeconomic conditions where labor-intensive practices either are economically feasible or economics is not a major factor. That partly explains the current decrease in their use. Use of free-living blue-green algae should not be as labor-intensive as green manuring, but has only moderate potential and is still limited by methodological problems. It is unlikely that  $N_2$  fixation could be an exclusive N source for attaining high rice yields under the most economical conditions. The future of biological  $N_2$  fixation in rice cultivation most probably lies in integrated management, but economically feasible techniques still have to be designed.

Numerous experiments have shown that broadcasting N fertilizer into floodwater leads to a wastage of N by 1) favoring  $NH_3$  volatilization, and 2) strongly reducing the free natural N input by photodependent  $N_2$  fixation. It was also shown that broadcasting N fertilizer favors the proliferation of mosquitoes. However deep placement of fertilizer is not widely practiced because of the lack of cheap and efficient methods and implements.

There is growing concern about the sustainability of high rice yields. Several long-term experiments in research stations have shown a yield decline with time (Flinn et al 1984, Pingali et al 1990). Flinn and De Datta (1984) reported declines of about 0.1 t/ha per yr for high-yielding entries between

**Table 8.1. Current status of the agricultural management of the ricefield floodwater ecosystem.**

Method	Status <sup>a</sup>	Area <sup>b</sup>	Remarks
<i>Control of detrimental algae and weeds</i>			
Microalgae scum			
with algicides	Farm	? (i)	In direct seeded rice only
with drainage	Farm	? (i)	In direct seeded rice only
Macrophytic algae with algicides	Farm	?	Significant in some areas
Algae in relation with ammonia volatilization			
fertilizer deep placement	Farm	?	Not broadly adopted
with algicides	Research		
Weed control with water fauna			
with fish	Farm	(1.5)	Secondary effect of rice-fish culture
with crustaceans	Research		Limited to transplanted rice
<i>Aquatic weeds as fertilizer</i>			
	Farm	?	Mostly in China <sup>c</sup>
<i>Biofertilizers</i>			
<i>Azolla</i>	Farm	1.2	Mostly in China <sup>c</sup> and Vietnam
Cyanobacteria			
Inoculation	Farm	0.5	Mostly in India
Promoting indigenous strains	Research		
<i>Aquaculture</i>			
Rice-fish culture	Farm	1.5	Mostly in China <sup>c</sup> and Indonesia
Rice-shrimp culture	Farm	? (i)	
<i>Control of aquatic fauna detrimental to rice</i>			
Snails		?	Significant in several countries
with molluscicide	Farm	?	
with management methods	Farm	?	Often combined with molluscicide
Other animals	Research		Incidental
<i>Control of vectors</i>			
By water management	Research		
Conservation of natural predators	Research		Secondary effect of IPM
By cultural practices	Research		Secondary effect of some practices
By biological control with fish	Farm	(1.5)	Secondary effect of rice-fish culture
with <i>Azolla</i>	Farm	? (i)	Gambusia in USA
with <i>Bacillus thuringiensis</i>	Farm	(1.2)	Secondary effect of <i>Azolla</i> use
Others	Research	? (i)	In USA

<sup>a</sup>Farm = currently used by farmers; Research = at research level only. <sup>b</sup>Rough estimates in 10<sup>6</sup> ha subject to caution; ? = unknown; ? (i) = unknown but incidental. <sup>c</sup>Excluding Taiwan Province.

1966 and 1980. Reasons for yield decline are still poorly understood. It may result partly from the combined effects of increased OM inputs as crop residues and the decreased or changed decomposition processes under extended periods of anaerobic conditions. Preliminary experiments have shown that PAB significantly contributes to the replenishment of soil microbial biomass and available N. Crop intensification, by decreasing PAB and the inputs of OM with low C-N ratio into the soil, might decrease soil microbial biomass and N fertility. It is possible that changes in floodwater and soil fauna are also involved in the process because of reduced translocation of nutrients to the deeper layer of soil. Currently these aspects are mostly hypothetical and deserve further study.

Crop intensification has replaced the diversity of food production observed in traditional ricefields by rice productivity. An important issue is to maintain high rice yield while preserving the ability of the ricefield ecosystem to produce additional sources of protein. Rice-fish culture is probably the most promising approach for an ecologically sound and economically successful management of the floodwater ecosystem. Agroecological interactions of rice-fish culture (control of rice pests and diseases, control of vectors, effect on soil fertility and rice yield, etc.) need to be clearly identified and quantified.

The impact of pesticides on ricefields is multifaceted. Misuse of pesticides has been one of the most serious environmental problems associated with the adoption of new rice technologies, resulting in poisonings, increases in pest and plant disease outbreaks, and in pesticide-resistant strains of rice pests (Oka 1988). It is recognized that flooded rice soil is an environment favorable for rapid detoxification of many pesticides, which may explain an absence of significant microbiological effect of pesticides in 73% of the tests performed in situ. However, there are reports of significant direct or indirect effects of pesticides on microorganisms, primary producers, and floodwater invertebrates important to soil fertility. A recent bibliographic and experimental assessment of their impacts on soil and water microflora and invertebrates concluded that current knowledge on wetland soils is too fragmentary to draw conclusions other than general trends (Roger et al 1992). Study of the effects of pesticides, hitherto mostly restricted to short-term laboratory conditions, must be performed on a long-term basis under more realistic field conditions and cultural practices.

A better understanding of the floodwater ecology is needed to develop agricultural practices that control vectors of human and animal diseases by conserving their indigenous invertebrate predators—which is a major component of IPM.

While there is an urgent need for increasing rice production, there is a similar need to identify and study ecological interactions that govern the sustainability of the irrigated ricefield resource system and the rice-growing environment so that environmentally safe and economically feasible technologies can be developed.

## 9 Appendix: methods for ecological studies in ricefields

This chapter summarizes the major methods of studying floodwater populations and activities in wetland ricefields. First, it considers the general characteristics of the distributional ecology of organisms and activities in ricefield floodwater and soil and the implications for sampling strategies and accuracy of the measurements. Then it provides the reader with a brief description of the methods together with bibliographic references for additional information.

### 9.1. Distributional ecology of organisms and activities: implications for sampling

The validity and accuracy of quantitative measurements of populations and activities in a ricefield depend on how representative the samples are of the whole population. That depends on the heterogeneity of the organisms' distribution and the method and density of sampling or subsampling. Therefore, before designing a sampling method, the distribution law of the organisms or activity must be characterized. This should determine the sampling strategy and the density of sampling needed to obtain a given representativeness of the samples.

#### 9.1.1. Distributional ecology of populations and their activities

Symbols and abbreviations used in this section are summarized in Table 9.1.

In soil biology, the most convenient method of determining the distribution law of a variable is to study the correlation between means and variances of several groups of replicated measurements performed at different times and at different sites using the same technique. When a variable is normally distributed, the mean ( $m$ ) and the variances ( $s^2$ ) are independent.

A correlation between mean and variances indicates a non-normal distribution, which is most often the case for organisms and their activities in aquatic or terrestrial environments. Their non-normal distribution is characterized by a linear correlation between means and variances of groups of replicated measurements. Plotted on a log-log graph, the points ( $m$ ;  $s^2$ ) exhibit a linear arrangement. That is known as Taylor's power law (Taylor 1961), that the variance ( $s^2$ ) of a population is proportional to a fraction power of the arithmetic mean ( $m$ ):

**Table 9.1. Statistical symbols and abbreviations.**

m	arithmetic mean of n values of a variable (x)
n	number of data ( $n_a$ : number of data for the variable $x_a$ )
$m_a$	arithmetic mean of $n_a$ values of a variable ( $x_a$ )
$s^2$	estimated variance of the mean (m) of n values of (x) when needed, we use
$s_x^2$	estimated variance of the mean (m) of n values of (x)
$s_y^2$	estimated variance of the mean of n transformed values (y) of (x) with $y = f(x)$ (most often $y = \log_{10}(x + 1)$ )
s	estimated standard error of the mean (m) of n values of (x) similarly we use, when needed, $s_x$ and $s_y$
t	Student-Fischer t variable
cv	coefficient of variation (%) = 100 s/m
CI	confidence interval is expressed as $m \pm \frac{ts_x}{\sqrt{n}}$
$L_u$ and $L_l$	upper and lower limits of the confidence interval
P	accuracy = CI/m
$P_e$	accuracy defined as half of the difference between the upper and lower confidence limits of the confidence interval, $P_e = \frac{1}{2} \frac{L_u - L_l}{m}$
p	probability level of statistical tests

$$s^2 = a m^b \text{ or } \log s^2 = \log a + b \log m$$

The intercept (a) and the slope (b) of the regression curve, on a log-log scale, are population parameters. The slope (b) is an index of the distribution law of the variable and determines the transformation that normalizes the distribution as follows:

- A slope (b) close to 1 characterizes Poisson distributions, which can be normalized by transforming the data by the equation

$$y = x^{1/2}.$$

- A slope between 1 and 2 characterizes negative binomial distributions, which can be normalized by transforming the data by the equation

$$y = x^{1-(b/2)} \text{ or } y = \log_{10}(x + x_0)$$

where  $x_0$  is a constant that can be determined by a graph method.

- A slope of 2 characterizes log-normal distributions (the values' logarithms are normally distributed), which can be normalized by transforming the data by the equation

$$y = \log_{10}x, \text{ or by } y = \log_{10}(x+1)$$

when there are  $x$  values equal to 0. Distributions with  $(m; s^2)$ -regression curve slopes between 1.7 and 2.4 can be considered as log-normal (Roger et al 1981). The standard deviation is close to the mean, and the CV of the data is close to 100%.

In ricefields, populations of algae (enumerations or biomass estimates), counts of soil and water invertebrates, and estimates of photodependent  $N_2$  fixation by acetylene-reducing activity (ARA) have most often an aggregative distribution that approximates a log-normal pattern. That distribution is observed for single-locus samples collected in the same plot and for single-locus and composite samples collected in replicated plots. The most common explanation of log-normal distributions is that small variations of normally distributed physicochemical factors in the environment induce exponential responses of the biological variables.

Exceptions to a log-normal distribution include 1) experimental plots with a bloom, because the small plot size limits algal growth, thus decreasing the variability between plots, and 2) algal counts of plowed soils where spores are more evenly redistributed. In those situations, a negative binomial distribution is observed. Populations of motile soil invertebrates exhibit negative binomial distributions more often than populations of microorganisms and algae.

*Selected references on distributional ecology of microflora, phytoplankton, and zooplankton:* El-Shaarawi et al 1981, Frontier 1973, Loper et al 1984, Roger and Reynaud 1978, Roger et al 1977, Taylor 1961.

### 9.1.2. Sampling strategy and measurement accuracy

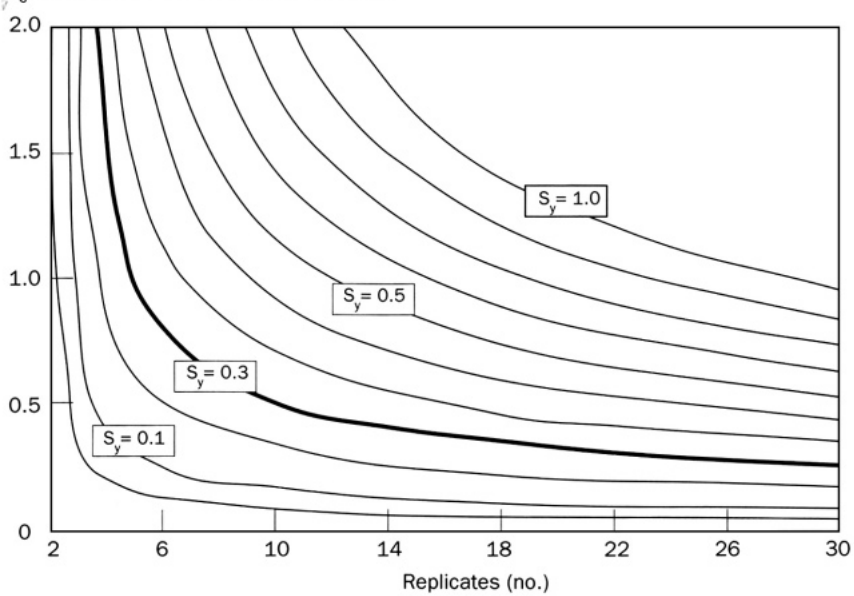
In log-normal distributions, the accuracy of the mean of  $n$  measurements ( $P_e$ ), defined as half of the confidence interval expressed as a fraction of the mean, is calculated as

$$P_e = \frac{1}{2} \left( 10^{\frac{tS_y}{\sqrt{n}}} - 10^{-\frac{tS_y}{\sqrt{n}}} \right) \quad (\text{Eq. 1})$$

where  $t$  is the statistic of Student-Fischer,  $n$  is the number of replicates, and  $S_y$  is the standard deviation of the logarithms of the data (Roger et al 1978).

Figure 9.1 presents a graphic representation of this function for values of  $S_y$  ranging from 0.2 to 1.0. When  $S_y$  estimates are available from previous measurements performed under similar conditions, this graph can be used to determine the number of replicated plots needed for a given accuracy, or the number of subsamples needed for a given representativeness of a composite sample within a plot.  $S_y$  values obtained from 653 groups of measurements of microbial and algal populations and activities measurements under a wide range of conditions ranged between 0.1 and 1, with a median of 0.32 (Roger et

$$P_e = 1/2 ((\text{upper limit} - \text{lower limit})/\text{mean})$$

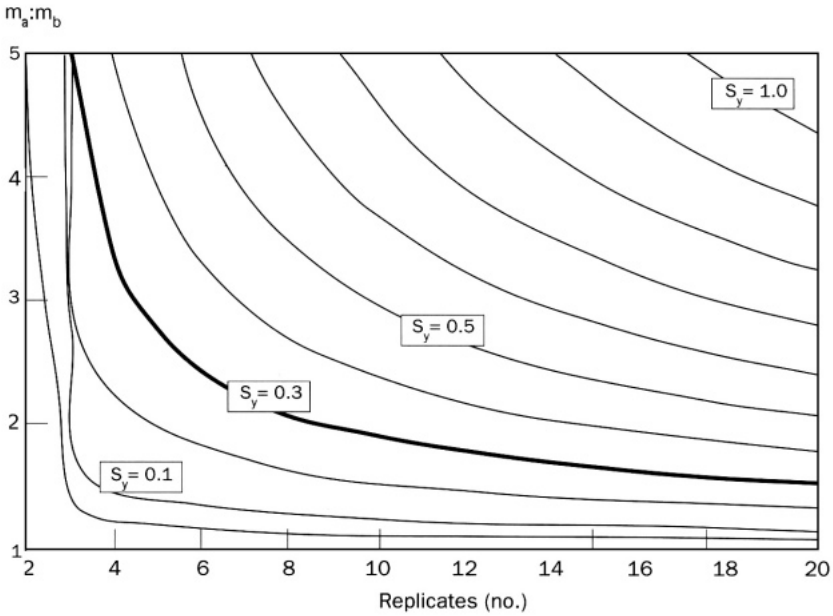


**9.1.** Accuracy ( $P_e$ ) of a mean of log-normally distributed data as function of the number of replicates ( $n$ ) and the standard error of the logarithms ( $S_y$ ) (Roger et al 1991).

al 1991a). Applying this value to Equation 1 indicates that the accuracy of averages of biological measurements in replicated plots is usually low. For example, measurements in 10 replicated plots provide an accuracy of 0.5, for which the confidence interval is equal to the mean (Fig. 9.1). Using composite samples markedly increases the representativeness of measurements within a plot. However, as Figure 9.1 shows, on the average ( $S_y = 0.32$ ) 10 core subsamples are needed for a representativeness of 0.5. A representativeness of 0.3 requires 26 cores, and a representativeness of 0.2 requires 55. These data show that the number of subsamples collected from a plot is often determined by methodological limitations (i.e., the maximum number of subsamples that can be reasonably collected or handled) rather than a chosen accuracy.

Rather than the accuracy of a mean, however, the major concern in field experiments is the number of replicates needed to establish a significant difference between two means  $X_a$  and  $X_b$ . Assuming that  $n_a = n_b$  and  $S_{y_a} = S_{y_b}$ , the ratio  $X_a : X_b$  required to establish a significant difference between  $X_a$  and  $X_b$  ( $p = 0.05$ ) is given by the equation

$$\frac{\bar{X}_a}{\bar{X}_b} = 10 \sqrt{\frac{2S_y^2}{n}} \cdot t_{(2n-2)} \quad (\text{Eq. 2})$$



**9.2.** Ratio ( $m_a:m_b$ ) between two means of  $n$  replicated measurements of a log-normally distributed variable ensuring a significant difference ( $p = 0.05$ ) between  $m_a$  and  $m_b$ .  $S_y$  = standard error of logarithms of the data (Roger et al 1991).

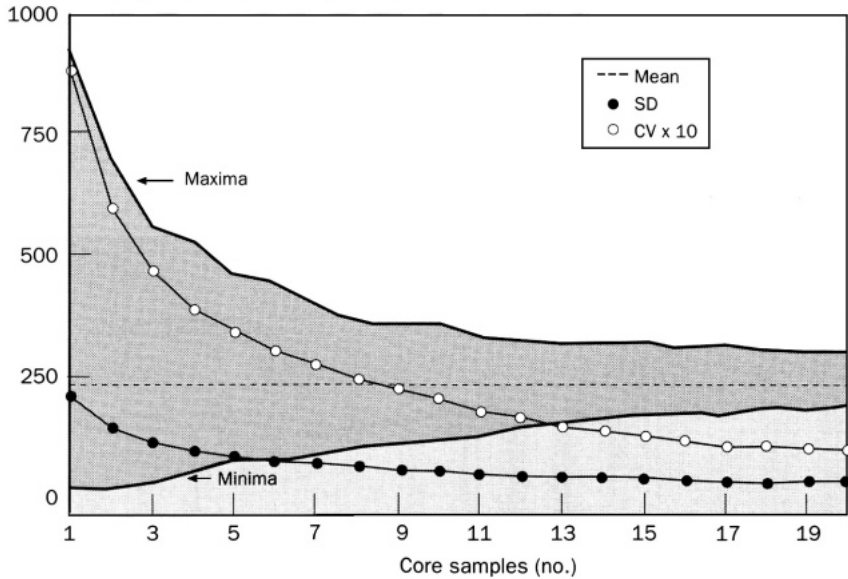
where  $n$  is the number of replicates,  $S_y^2$  is the variance of transformed data, and  $t_{(2n-2)}$  is the  $t$  value of Student-Fischer statistic at  $(2n-2)$  degrees of freedom (Roger et al 1978). Figure 9.2 presents calculations of  $X_a:X_b$  for  $2 < n < 20$  and  $0 < S_y^2 < 1$ .

The values of  $X_a:X_b$  corresponding to the median value of  $S_y$  (0.32) show that, on an average, 5 replicated plots will only show significant differences between values whose ratio is about 3. Ten replicates would establish a significant difference between two values whose ratio is 2. Most field experiments on rice usually have three or four replicates, which is adequate for normally distributed data such as rice yield, but often might be insufficient for estimating some biological variables with an acceptable accuracy.

A coefficient of variation of 100% is observed among single-locus measurements because of their log-normal distribution. The most efficient method to decrease the variability of measurements within a plot is to replace individual samples by composite samples, which changes the log-normal distribution of the data to a normal distribution if the number of subsamples is high enough. This property is known as the central limit theorem.

Figure 9.3 presents the average, maximum, minimum, standard deviation, and CV of sets of 230 estimates of ARA in a single plot, calculated by

ARA ( $\mu\text{mol C}_2\text{H}_2/\text{m}^2$  per h) and CV (%  $\times 10$ )



**9.3.** Mean, maxima, minima, standard deviations (SD), and coefficients of variation (CV) of 230 estimates of acetylene-reducing activity (ARA) in a  $16\text{-m}^2$  plot calculated by random selection of 2-20 values from among 35 single-locus measurements within the plot (Roger et al 1991).

random selection of 2-20 values from among 35 values measured on 35 single soil cores collected in a  $16\text{-m}^2$  plot. It shows that the CV of 100% observed with the log-normal distribution of single-locus measurements rapidly decreases together with the dissymmetry of the upper and lower values when the number of subsamples increases. Because of the rapid decreases of the CV as the number of subsamples increases, the accuracy of the mean of measurements on composite samples in replicated plots depends mainly on the number of plots. In particular, it does not improve when the value of the accuracy of the measurements in each plot decreases beyond the value of the accuracy that can be expected from the number of replicated plots. Therefore, the determination of the optimum number of subsamples to be collected in a plot should take into account accuracy or representativeness for the lower limit and the number of replicated plots for the upper limit.

These data show the importance of sampling strategies in performing field measurements. Such measurements are often extremely tedious and an adequate sampling and measurement strategy can avoid either superfluous sample collection or measurements—that will not improve significantly the accuracy of the data—or measurements that have little chance to produce conclusive results.

*Selected references on statistical implications of the distributional ecology of organisms in ricefields:* Roger et al 1981, Roger and Reynaud 1978, Roger et al 1978, Roger et al 1991a.

### **9.1.3. Sampling procedures**

Most aquatic organisms studied in ricefields develop both in the water and the upper soil layer. The easiest method of gathering floodwater and soil in one sampling operation is to collect core samples that include floodwater and soil to the desired depth. A core of 2-3 cm diam enables easy collection and permits use of many subsamples, which increases the reproducibility of the composite sample measurements. After collection, cores can be processed as a whole or separated into floodwater, surface, and deep soil.

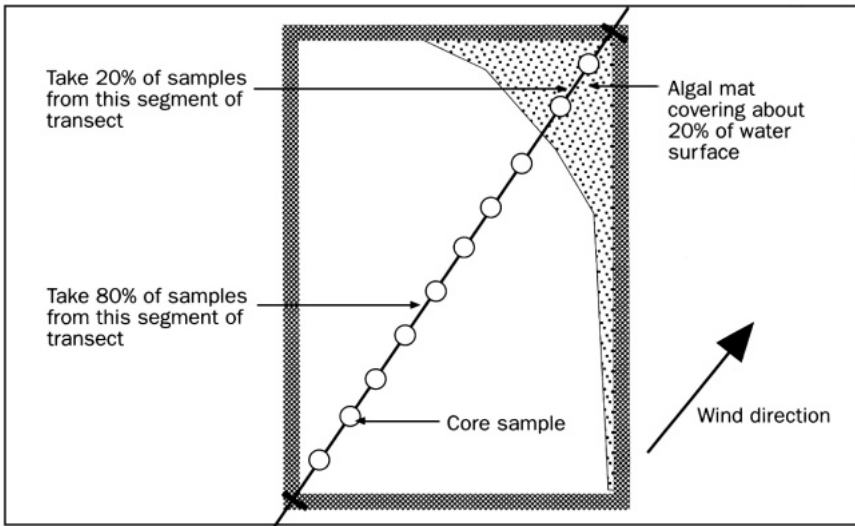
Core sampling enables the determination of population densities on both volumetric and area bases. But the expression of densities in volumetric terms (often used in freshwater bodies) is intrinsically erroneous for ricefields. Floodwater depth is controlled by the combined affects of irrigation, evapotranspiration, percolation, and rainfall. When depth increases, populations are diluted and vice versa. The life cycles of floodwater inhabitants are too long for there to be a response to continually changing depth. Area remains constant, therefore the expression of population densities in spatial terms (organism or activity/cm<sup>2</sup>) is more realistic and allows extrapolations and comparisons by field area.

Core sampling procedures for wetland soils are described in sect. 9.2.11.

Algae, aquatic plants, and soil fauna are usually irregularly distributed within a plot. Because of the uneven distribution, random sampling is definitely the worst sampling method. Sampling strategy must be designed after careful examination of the plot and must be adapted to the specific situation.

In experimental plots, a transect sampling strategy is preferred to random sampling for the following reasons. First, in small plots, random sampling would give a disproportionately large weight to the plot's perimeter compared with that expected in a full-sized field. Second, when large numbers of plots are to be sampled, it is beneficial to have an easily reproducible method, which involves minimum organization in the field. Assigning random locations to all cores in many plots is logistically tedious. Third, sampling along transects rather than at random minimizes disturbance of the plots.

When no algal growth is visible, take samples at regular intervals on a transect through the plot. If few colonies are floating on the water surface, collect some for identification, but do not collect them in the cores when sampling for ARA measurements or counts. When patches of algal mat float on or partially cover the water surface, visually evaluate the percentage of area covered by the mat and take the same percentage of cores from within the mat. Dominant directional wind pushes floating mat(s) in one direction and leads to a gradient of algae and algal propagules in the plot. Sample along a transect that passes through the center of the plot and is oriented toward the domi-



9.4. Sampling method.

nant wind direction (Fig. 9.4). When sampling for zooplankton, use a method of sampling that prevents organisms from escaping.

Process fresh samples as soon as possible. Populations of microalgae and cyanobacteria can change rapidly, especially if algal grazers (snails, ostracods, etc.) are collected in the tube.

In dry soils, when core sampling is usually not possible, collect subsamples by delineating areas with a core borer and removing the upper 0.5 cm layer of soil with a knife blade. Dry soil samples are usually used only for enumerating algae, so the final size of the composite sample is not a limiting factor. Therefore, a larger core borer (3-5 cm diam) can be used, which facilitates sampling.

## 9.2. Sampling methods for algae and aquatic plants

### 9.2.1. Qualitative study of the algal flora

Many ecological studies of algae in ricefields have simply recorded and identified species (Roger and Kulasooriya 1980). The studies often employed the liquid culture method, which involves inoculating a small quantity of soil in an Erlenmeyer flask. Algae are sampled and identified at intervals. Because of medium selectivity and competition between strains, this easily employed method does not ensure that all species present in the soil will grow in the flask, resulting in underestimation of algae occurrence and diversity. It is now well established that  $N_2$ -fixing cyanobacteria are ubiquitous in ricefields, but early studies reported few  $N_2$ -fixing cyanobacteria because their findings were

derived through the liquid culture method (Watanabe 1959, Watanabe and Yamamoto 1971, Venkataraman 1975).

Plating soil suspension-dilutions on agar medium (see sect. 9.2.8) permits a more complete record of strains present in a soil and allows easier isolation and identification than the liquid culture method. It also allows quantitative estimation of the populations. We do not recommend the liquid culture method, or qualitative studies in general, because more useful information can be obtained from quantitative studies for relatively little additional effort.

Algae abundance in soil has been determined by

- direct and indirect biomass estimates;
- measurement of pigments;
- direct observation and counts;
- indirect counts using the serial inoculation of soil suspension-dilutions in tubes of media and calculation of the most probable number from a record of the tubes showing algal growth; and
- serial plating of soil suspension-dilutions on a solidified medium in Petri dishes, and counting colonies after 3-4 wk of growth.

### **9.2.2. Estimation of aquatic macrophytes and algal biomass by direct measurements**

Collecting aquatic macrophytes or algae in a representative area of a plot is the easiest and most efficient way to estimate aquatic plant biomass. For microalgae it is possible only when a bloom develops. Sampling precautions should be taken to ensure reliable measurements of the algal biomass. A composite sample should be collected when most algae are floating; avoid sampling in the early morning.

Depending on the algal distribution, collect subsamples at regularly distributed sites, along a transect, or at chosen sites (see sect 9.1.3). In a 4 × 4-m plot, we usually collect algae from 16 squares delineated by 4 rice hills. A plastic frame (i.e., a bottomless plastic tray) facilitates sample collection. To collect floating algae, we use a 15-cm-diam metallic screen attached to a 2-m handle. Combine and mix the 16 subsamples before measuring.

Collect epipellic algae (and macrophytic algae) by hand. The biomass of epipellic algae is often lower than that of floating algae. It is best not to try measuring epipellic algae when they are not very abundant because they cannot be harvested without simultaneously collecting large quantities of soil, which interferes with the measurements.

To provide information on the agronomic significance of the photosynthetic aquatic biomass (PAB) as fertilizer, measurements should include determination of fresh weight (fw), dry weight (dw), N, and possibly ash, C, and P contents because the composition of ricefield algae and cyanobacteria is highly variable. A bloom of agronomic significance (corresponding to at

least 5 kg N/ha) usually has a biomass greater than 10 t fw/ha, and is therefore visible to the naked eye.

### 9.2.3. Estimation of algal biomass by the square count method

Rice plants in transplanted plots provide a grid that can facilitate the visual recording of algal growth. In 4- × 4-m plots, one may rapidly and systematically examine the floodwater surface and record the number of squares delineated by four rice hills where algae are present. In a given ricefield, there is usually a good correlation between the percentage of squares with floating algal colonies and the algal biomass. This square count method requires case-to-case standardization because the regression between percent of squares with algae and total algal biomass depend upon the type of algae and algal growth. But the method is less tedious and much faster than some direct measurements.

As with any visual method, the operator's skill determines the accuracy of records. CVs of counts by 4 independent, untrained operators in 16 plots ranged from 10 to 50% and averaged 24%, whereas CVs of counts by 3 trained operators ranged from 2 to 33% and averaged 11% (Roger et al 1991a).

We recommend the square count method when algae are not abundant. When colonies or patches are numerous or when a bloom develops, an index of abundance is faster than the square count method.

### 9.2.4. Estimation of algal biomass by an index of abundance

Different indices of abundance can be used, but simple scores (0-5) are subjective, provide limited information, and do not allow quantitative estimation of algal biomass.

We use a semiquantitative index based on estimating how much of the floodwater surface area is covered with algae:

Status of algal growth	Code	Index	Index (%)
No algae visible	0	0	0
Algae present with a few colonies	+	1	5
Algae present	++	2	10
Algae present	+++	3	15
Algae present	++++	4	20
Algae covering 1/4 of the plot	1/4	5	25
Algae covering 1/3 of the plot	1/3	6	33
Algae covering 1/2 of the plot	1/2	7	50
Algae covering 2/3 of the plot	2/3	8	67
Algae covering 3/4 of the plot	3/4	9	75
Algae covering the whole plot	All	10	100

This index allows a rough estimate of the biomass when it has been standardized previously from a harvested algae crop. The accuracy depends on the operator's skills and may vary significantly between operators.

Visual methods are less accurate than direct measurements and are difficult to use if the rice canopy is dense. However, when carefully applied, visual methods are useful to test the effects of treatments on the standing algae crop. Because algal biomass varies widely between replicated plots, visual methods provide the same information as direct measurements, especially during the first 30-50 d of the crop cycle, and require much less work.

### **9.2.5. Estimation of algal biomass by pigment measurement**

Pigment analysis, especially the determination of chlorophyll a, is used frequently in limnological studies. Results from soils, however, are affected by acetone extracting colored organic substances such as humic acids and chlorophyll degradation products (e.g., phaeophytins and chlorophyllides) from the samples. Therefore, the pigment measurement method can only be used to compare the effect of different treatments on the total algal biomass in the same soil. Because the same information can be obtained more easily by measuring dissolved O<sub>2</sub> we do not recommend pigment measurement.

### **9.2.6. Estimation of algal abundance by direct counts**

Direct examination and counting are most frequently used in hydrobiological studies. Algae from a water sample are concentrated, fixed, counted, and identified using an inverted microscope. Direct counts have also been performed on soil suspensions (Tchan 1952, 1953; Brock 1978). But the method is tedious and time consuming, soil particles and aggregates in the suspension hinder counting, and algal filaments break, making identification difficult. The method is slow except when the algal population is dense. It does not allow isolation of the strains and is often inappropriate for simultaneous studies of many soil samples. Therefore we do not recommend direct counts.

### **9.2.7. Estimation of algal abundance by the most probable number method**

The most probable number (MPN) method has been most frequently used to enumerate algae in soils because it requires much less work than the plating method. The MPN method, however, often underestimates algal abundance compared with plating counts. Lower MPN values are partly due to less favorable conditions for algal growth in tubes, where CO<sub>2</sub> diffusion in the medium and light availability are less than in Petri dishes. In addition, the MPN method provides little information on the qualitative composition of the algal flora. The species recorded in the last positive dilution will have the most colony-forming units (CFU)/ml. This species is often not dominant in terms of biomass, but is a small-celled species such as a unicellular cyanobacteria or *Pseudanabaena*. Therefore, we do not recommend MPN methods for algae counts in soils.

### 9.2.8. Estimation of algal abundance by the plating method

Plating is the most useful method for counting soil algae. Its use in ricefields was discussed by Roger and Reynaud (1978), Reynaud and Laloe (1985), and Roger et al (1991a). The standardized procedure is composed of the following steps:

1. Collect a composite sample of surface soil; include the corresponding floodwater if the soil is submerged.
2. Prepare a  $10^{-1}$  dilution based on surface area.
3. Prepare and plate subsequent dilutions ( $10^{-2}$  to  $10^{-6}$ ) on Petri dishes.
4. Incubate for 3 wk under standardized conditions.
5. Count under a stereoscopic microscope and identify under an optical microscope.

*Sampling.* The composite sample is composed of at least 10 core subsamples (each about 2 cm diam), including the top 0.5 cm of soil, and, if the soil is submerged, the corresponding floodwater. Dry samples are ground to  $< 1$  mm, but drastic grinding must be avoided because it may decrease the counts.

*Preparation of dilutions.* Use core samples to prepare the suspension dilutions. Adjust the volume of the first soil suspension-dilution with distilled water to a value 10 times the  $\text{cm}^2$  value of the total surface area of the collected core samples. That provides the required  $10^{-1}$  dilution/surface area. For example, if 10 core samples, 2 cm diam are collected, the total area sampled is  $1 \times 1 \times 3.14 \times 10 = 31.4 \text{ cm}^2$ . The first dilution is adjusted to a 314 ml total volume. One 1 ml of this  $10^{-1}$  suspension-dilution is equivalent to  $10^{-1} \text{ cm}^2$  of soil.

When sampling on a dw basis (dry soils, soil based inocula, etc.), prepare the  $10^{-1}$  dilution by suspending 10 g soil in 90 ml distilled water.

Stir the first dilution at 400 rpm for 30 min to disrupt algal clumps. Dilute serially. Stir subsequent dilutions ( $10^{-2}$ – $10^{-6}$ ) for 1 min before subsampling. Plate dilutions from  $10^{-2}$ , to  $10^{-6}$  using three replicates/dilution.

*Plate preparation.* The total algal flora is evaluated on 1% solidified BG-11 medium (Stanier et al 1971) containing mineral N. This medium is suitable for many unicellular chlorophyceae, diatoms, and cyanobacteria. Use the same medium, depleted of  $\text{NaNO}_3$ , to enumerate  $\text{N}_2$ -fixing cyanobacteria. Dry plates for 2 d before plating and then use or store in sealed plastic bags.

*Incubation.* Incubate Petri dishes for 3 wk at laboratory temperature (22–30°C) under continuous light (about 800 lx) from cold white fluorescent lamps. To save shelf space, pile the three replicates of the same dilution in an inverted position 2 d after plating, and interchange them every 2–3 d to ensure similar illumination of the replicates. Counts may be performed after 2 wk incubation for eukaryotic algae and non- $\text{N}_2$ -fixing cyanobacteria. For enumerating  $\text{N}_2$ -fixing cyanobacteria, 3 wk are needed to deplete N traces from the so-called *N-free* medium, and to bleach non- $\text{N}_2$ -fixing strains, which facilitates counting of  $\text{N}_2$ -fixing cyanobacteria.

*Counts.* Observations and counts are usually done at two consecutive dilutions:  $10^{-3}$  and  $10^{-4}$ , or  $10^{-4}$  and  $10^{-5}$ . At the higher dilution, there are fewer colonies, which can be easily observed and transferred to an ordinary microscope for identification. The lower dilution is used primarily to obtain quantitative data. Place the Petri dish on a transparent square screen with 1 cm mesh and count colonies under a stereoscopic microscope. An operator who is familiar with major colony types can count them with the naked eye, especially if colonies are large and well individualized. Counts taken with the naked eye, however, tend to underestimate values by omitting small colonies. On the other hand, counts under the stereomicroscope may overestimate values if some colonies are counted twice, or they may underestimate the number of large and diffuse colonies.

*Interpretation and expression of results.* Because of competition on Petri plates, counts at 2 consecutive dilutions are in the ratio of about 6 rather than in the expected ratio of 10 (Roger et al 1991a). To avoid a marked effect of competition, use data from dishes with from 3 to 30 colonies. The 30 value allows significant colony counts, but is low enough to avoid marked competition. This number may be increased when colony sizes are small ( $< 1$  mm) or decreased when colony sizes are large ( $> 5$  mm). However, selecting dishes with less than 30 colonies, together with the lower-than-theoretical value (10) of the ratio between counts at two consecutive dilutions, causes a bias in the relative frequencies of the first digit of algal counts. The relative frequencies of the first digit (1-9) of algal counts correlate negatively with the value of the digit. Lower digits occur more frequently than higher ones, although an even distribution is expected.

Depending on whether core or bulk sampling methods are used, counts are expressed as CFU/cm<sup>2</sup> or CFU/g dw of soil. Expressing the results on a unit area is preferable because it enables extrapolations to CFU/ha. Data expressed in CFU/g dw of soil preclude extrapolations to area because of the vertical variation of cyanobacteria propagule density in the soil. When extrapolations must be done from surface soils sampled by dw, results can be roughly converted to area by assuming that 1 cm<sup>2</sup> of soil is approximately equivalent to  $0.3 \pm 0.1$  g dw (Roger et al 1991a).

*Reproducibility and accuracy.* The reproducibility of the method (intraplot variability), tested from 30 composite samples (each consisting of 10 cores) from a 16-m<sup>2</sup> plot, showed that the representativeness (1.96 s expressed as a % of the mean) of a single enumeration is about 60%. Also, two single enumerations cannot be considered significantly different if the ratio between them is less than 4. The plating method is tedious and time consuming, thus, counts frequently are not replicated, but it does not require many replicates to show significant differences or changes between populations.

*Advantages and disadvantages.* Plating soil suspension-dilutions has one major advantage—it is the only method that enables simultaneous enumera-

tion, identification, and isolation of algae present. Three major limitations are as follows.

- The plating method uses artificial media, which may result in some strain selection and may not ensure the development of all species present. Relative growth frequencies may be altered.
- The method does not distinguish actively growing cells or filaments from spores or propagules dormant in the soil. It probably favors spores and propagules, because they are little affected during the preparation of soil suspension-dilutions and find conditions favorable for their growth in Petri dishes. Actively growing cells, however, might be negatively affected by 1) osmotic shock during the preparation of soil suspension-dilutions, and 2) the changes in environmental conditions they are subjected to both during and after plating.
- Because of competition between too many colonies, a bias is introduced in the numerical values, and strains present at densities lower than 1% of CFU are usually not recorded. Thus the method is suitable only for quantitative estimation of the major strains present.

*Selected references on plating method:* Reynaud and Laloe 1985, Roger and Reynaud 1978, Roger et al 1991a.

*Selected references reporting culture media for microalgae and cyanobacteria:* Allen and Arnon 1955, Gerloff et al 1950, Gorham et al 1964, Kratz and Myers 1955, Reynaud and Roger 1977, Stanier et al 1971, Van Baalen 1965.

### **9.2.9. Selecting an appropriate method for estimating algal abundance**

There is no universal method for determining algal abundance in ricefields. The choice of method depends on the state of the field, the purpose of the measurement, and, in many instances, labor availability. Table 9.2. compares the suitability of various methods for different variables and environmental situations.

For aquatic macrophytes, direct biomass measurements are best. They can be partly replaced with the record of an index of abundance, preferably performed by several operators, if measurements are conducted at regular intervals during a crop cycle.

In soils with no visible algal growth, we recommend the plating method, using a composite sample collected on an area basis. To compare different soils, collect only the first 0.5 cm of soil. Deeper soil samples may be collected for estimating dormant spores or propagules in the soil. The plating method can also be used in fields with visible algal growth, especially to study the fate of an inoculated strain. For a quantitative estimate of the algal biomass, direct biomass measurements combined with visual records of abundance are much more accurate and informative and are less tedious than plating.

Measuring biomass from cell numbers and size is time consuming and liable to subjective errors in volume determinations. An algal biomass of ag-

ronomic significance is visible to the naked eye and, in most cases, can be estimated by direct measurements.

In flooded ricefields, when algal growth is moderate and the rice canopy is not too dense, the most convenient way to estimate algal biomass is the square count method combined with direct measurements using a quantitative composite algae sample. When algae are abundant or a bloom develops, direct biomass measurements are best combined with the record of an index of abundance, preferably performed by several operators. These methods should be combined with the collection of a composite sample to identify dominant strains and determine fw, dw, and ash, N, C, and P contents.

Of the available methods for quantitative estimation of algal populations, only plating soil suspension-dilutions enables simultaneous enumeration, identification, and isolation of most algae present. The method does not, however, distinguish actively growing cells or filaments from spores or propagules dormant in the soil, and uses an artificial medium, which may result in some strain selection. Because of competition between colonies on Petri dishes, quantitative estimates are biased and strains present at densities lower than 1% of CFU are usually not recorded. Therefore, the plating method is suitable only for quantitative estimation of the major strains present.

#### **9.2.10. Estimation of primary production by dissolved O<sub>2</sub> measurement**

To estimate gross primary production in ricefield floodwater, the method adapted by Saito and Watanabe (1978) from Odum (1956) is simple in that it requires only an oxymeter (YSI Model 57 dissolved oxygen meter was satisfactorily used in our studies). Dissolved O<sub>2</sub> and water temperature are measured at least every 2 h for a 24-h period (Fig. 9.5a). The O<sub>2</sub> probe is first calibrated in air, then it is dipped into the floodwater with slow and careful manual stirring until a stable output (ppm O<sub>2</sub>) is read. Four measurements are taken from each 16-m<sup>2</sup> plot. The floodwater level is also recorded.

Calculation of the gross primary production uses the following equations and calculations where

Q = rate of change of dissolved O<sub>2</sub> (DO<sub>t</sub>) per unit area (g O<sub>2</sub>/m<sup>2</sup> per h),

q = rate of change of dissolved O<sub>2</sub> (DO<sub>t</sub>) per unit volume (mg O<sub>2</sub>/liter per h),

P = rate of gross primary production per unit area (g O<sub>2</sub>/m<sup>2</sup> per h),

p = rate of gross primary production per unit volume (mg O<sub>2</sub>/liter per h),

R = rate of O<sub>2</sub> consumption per unit area (g O<sub>2</sub>/m<sup>2</sup> per h) [considered constant for a 24-h period],

r = rate of O<sub>2</sub> consumption per unit volume (mg O<sub>2</sub>/liter per h) [considered constant for a 24-h period],

D = rate of O<sub>2</sub> diffusion per unit area = ks (g O<sub>2</sub>/m<sup>2</sup> per h),

d = rate of O<sub>2</sub> diffusion per unit volume = ks (mg O<sub>2</sub>/liter per h),

DO<sub>sat</sub> = saturated dissolved O<sub>2</sub> at a given temperature [found in chemical handbook tables],

DO<sub>t</sub> = dissolved O<sub>2</sub> at a given time,

**Table 9.2. Comparison of the suitability of the different methods for characterizing/quantifying populations of microalgae in ricefields (After Roger et al 1991a).<sup>a</sup>**

Method	Record of species		Biomass estimation	
	Qualitative	Quantitative	Relative	Absolute
Liquid culture	Underestimates no. of species; provides limited information	NA	NA	NA
Biomass harvest	Microscopic observations on harvested material	NA	For moderate to dense algal growth (r)	For moderate to dense algal growth (r) For aquatic macrophytes (R)
Square counts	NA	NA	For sparse algal growth (r)	For sparse algal growth; requires standardization (r)
Index of abundance	NA	NA	(R)	Requires standardization (R)
Pigment measurement	NA	NA	Index of abundance or O <sub>2</sub> measurement provides same information with less work (NR)	NA
Direct counts	Does not allow strain isolation (NR)	Time consuming (NR)	(NR)	Requires standardization (NR)

Most probable number	Only the most abundant species is recorded (NR)	Underestimates algal populations (NR)	Underestimates algal populations (NR)	(NR)
Plating	Allows isolation of major strains (R)	Allows isolation of major strains (R)	Other methods provide same information with less work (NR)	Requires standardization (NR)
Dissolved O <sub>2</sub>	NA	NA	(R)	Requires 24 h measurements and standardization

<sup>a</sup>NR = not recommended, r = recommended for specific cases or with limitations, R = recommended, and NA = not applicable.

$Z$  = depth of the floodwater, and

$k$  =  $O_2$  diffusion constant.

The calculation is based on the following equations. During the day,

$$Q = P - R + D, \text{ and} \quad (\text{Eq. 1})$$

$$q = p - r + d = Q/Z. \quad (\text{Eq. 2})$$

Photosynthesis does not occur during the night and  $p = 0$ . Equation 2 then becomes

$$q = d - r. \quad (\text{Eq. 3})$$

The rate of  $O_2$  diffusion is proportional to the degree of  $O_2$  saturation of the water ( $s$ ):

$$d = ks \text{ and} \quad (\text{Eq. 4})$$

$$s = (DO_{\text{sat}} - DO_t) / DO_{\text{sat}}. \quad (\text{Eq. 5})$$

From Equations (3) and (4), we obtain

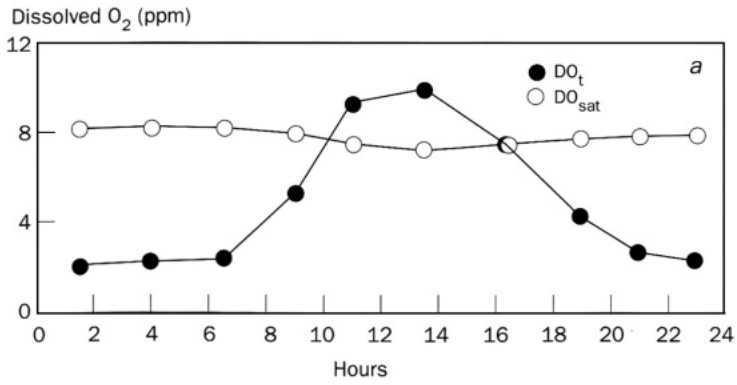
$$q = (k \times s) - r \quad (\text{Eq. 6})$$

at nighttime, which makes it possible to calculate  $r$  and  $k$  from DO measurements taken at night.

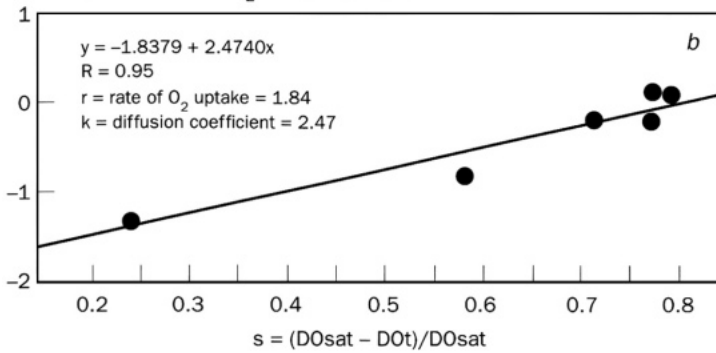
Assuming that the rate of  $O_2$  consumption (respiration and chemical consumption by reducing substances) is constant for a 24-h period, the diffusion coefficient ( $k$ ) is estimated by plotting  $q$  against  $s$  for the night measurements (Fig. 9.5b). Then records of DO (Fig. 9.5a) are used to calculate the rate of DO change ( $q$ ), which is then corrected for diffusion according to the values of  $k$  and  $s$ . The corrected value of  $q$  is plotted against time (Fig. 9.5c) and daily primary production on a volume basis is estimated from the cross-hatched area. Primary production is then expressed per unit surface area taking into account water depth.

The method is time consuming in that it requires an operator in situ every 2 h for 24 h. There is, however, a satisfactory correlation between primary production measured by 24-h records of dissolved  $O_2$  and the dissolved  $O_2$  measured at the time of maximum photosynthetic activity in floodwater, which occurs around noon (Fig. 9.6). Therefore, when establishing the dynamics of primary production in the floodwater of a field for a crop cycle, most of the 24-h measurements can be replaced by a single measurement.

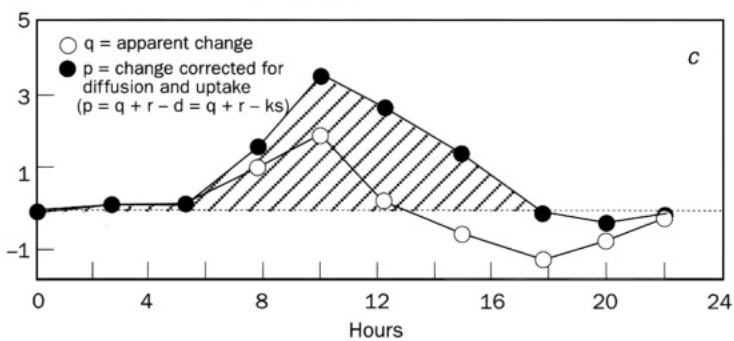
*Selected references on primary production measurement:* Odum (1956), Saito and Watanabe (1978), Vollenweider (1969).



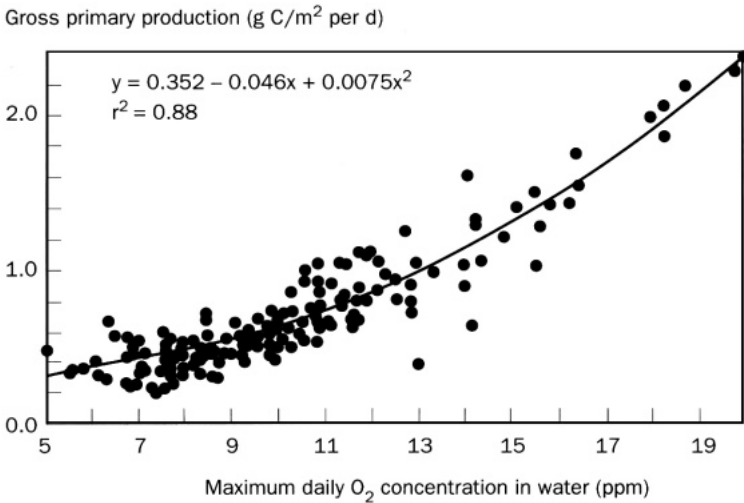
q: change of dissolved O<sub>2</sub> at night (ppm/h)



Rate of dissolved O<sub>2</sub> change (ppm/h)



**9.5.** Principle of the calculation of apparent daily primary production in ricefield floodwater from dynamics of O<sub>2</sub> concentration during the day.



9.6. Correlation between apparent daily primary production in ricefield floodwater and O<sub>2</sub> concentration in floodwater at 1300 h.

### 9.2.11. Estimation of biological N<sub>2</sub> fixation

There are three possible approaches to estimate biological N<sub>2</sub> fixation (BNF) during a crop cycle. The first is by classical N balance studies, which usually underestimate the value of BNF during the crop cycle, because N losses are not recorded. The method is not convenient for estimating in situ the contribution of N<sub>2</sub>-fixing phototrophs. The second possibility is to perform short-term measurements at regular intervals during the crop and to integrate them. That approach could be used with ARA (Hardy et al 1973) and short-term <sup>15</sup>N incorporation measurements to determine BNF by specific agents or groups of organisms. Currently, only ARA has been used for field studies at the crop-cycle level. The third approach is to determine the maximum biomass and the percentage of N derived from air (%Nd<sub>fa</sub>) of the N<sub>2</sub>-fixing agents.

*Methods using <sup>15</sup>N.* Methods using <sup>15</sup>N are not widely adopted for measuring BNF in ricefield floodwater and surface soil.

<sup>15</sup>N incorporation is not adapted to field measurements. It can, however, be used for short-term studies to assess BNF by various agents, to identify active sites in soil or water, and to establish the C<sub>2</sub>H<sub>2</sub>:N<sub>2</sub> conversion factor in water and soil samples (Watanabe and Roger 1985b; Eskew 1987).

Determining the maximum biomass and the %Nd<sub>fa</sub> by the <sup>15</sup>N dilution or the δ<sup>15</sup>N method is valid when the organism under study builds its maximum biomass with little turnover. In the case of cyanobacteria, which have a rapid turnover, the method may indeed lead to a marked underestimation of N<sub>2</sub> fixed. In addition, <sup>15</sup>N dilution trials with aquatic N<sub>2</sub> fixers such as *Azolla* and cyanobacteria have shown that fast changes in <sup>15</sup>N enrichment in the

water over time (Eskew 1987)—due in particular to losses by  $\text{NH}_3$  volatilization—result in large errors in %Ndfa estimation (Witty 1983). These problems can be solved by the sequential addition of  $^{15}\text{N}$  in water (Kulasooriya et al 1988) in the case of *Azolla*. But with cyanobacteria, the N level in water sufficient for growth of non- $\text{N}_2$ -fixing control algae may inhibit cyanobacteria growth directly or through competition.

The  $^{15}\text{N}$  method, which is based on the fact that soil has a higher  $^{15}\text{N}$  than air and therefore can serve as a naturally labeled medium, is considered suitable only for semiquantitative estimations. Its potential for quantitative measurement, however, has yet to be fully explored.

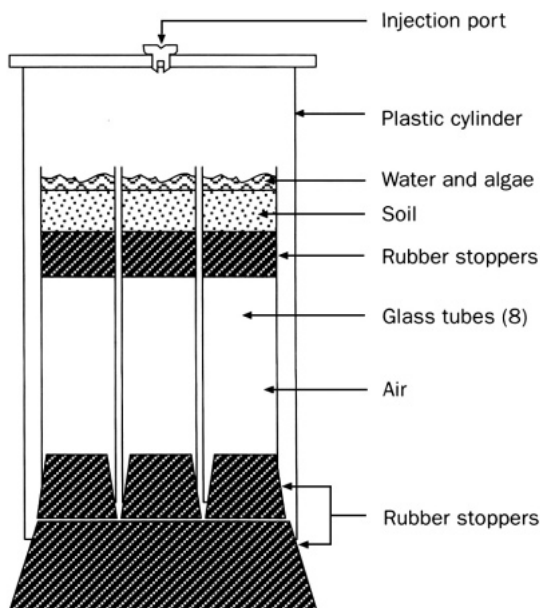
*ARA measurements.* Many variations of the ARA method have been used for in situ studies in ricefields and the associated methodological problems were reviewed by Watanabe and Cholitkul (1979). Major problems for estimating BNF in floodwater and surface soil from ARA measurements are as follows.

- ARA is not linear with time with cyanobacterial BNF.
- Most incubations are done under 10%  $\text{C}_2\text{H}_2$  in air, but ARA increases with up to 25%  $\text{C}_2\text{H}_2$  in air with thick cyanobacteria blooms.
- In situ, the greenhouse effect in enclosures used for incubation reduces photodependent ARA.

The  $\text{C}_2\text{H}_2:\text{N}_2$  conversion factor may vary from 3.9 to 30 with dense cyanobacterial mats. However, the variation depends mostly on  $\text{P}_{\text{N}_2}$  used for incubation under  $^{15}\text{N}_2$ . When  $\text{P}_{\text{N}_2}$  is similar to that of air, and all other factors are similar to those used for  $\text{C}_2\text{H}_2$  exposure,  $\text{C}_2\text{H}_2:\text{N}_2$  ratio is close to the theoretical value of 4 (Roger and Ladha 1992).

We describe hereafter a method for overcoming some of those limitations, one that has been used for most photodependent ARA measurements conducted in long-term experiments on the IRRI farm. Indeed many variations exist or can be derived.

Eight core samples are collected from each 16- $\text{m}^2$  plot with glass tubes (2 cm diam, 10 cm long). While collecting cores, the operator must minimize disturbance of the soil and of the ecosystem. To avoid muddying the waters, which affects measurements of photosynthetic ARA, the operator should stand on a dike or a board placed across the plot while inserting the glass tubes into the soil with minimum disturbance. To remove each tube with minimum disturbance of the ecosystem, first place a rubber stopper into the top of the tube, then rotate the tube to enlarge the hole in the soil, and finally, but only if necessary, support the soil with a finger while removing the tube. Tubes are then quickly stoppered at the bottom while unplugging the top. Soil cores in glass tubes are brought to the laboratory where floating algae and cyanobacteria, when present, are allowed to settle before removing the floodwater by suction to minimize problems of gas diffusion in water. To ensure better exposure of the algae and surface soil to the atmosphere of incubation,



**9.7.** Incubation device for photodependent ARA measurements with soil cores.

the soil is pushed to the top of the tube. Dead volume is prevented by plugging the bottom with rubber stoppers (Fig. 9.7).

For incubation under  $C_2H_2$  the 8 cores from each plot are held together with a rubber band and enclosed in a Plexiglas cylinder, 16 cm long and 7.5 cm diam, which is sealed with a Plexiglas plate on top and a big rubber stopper at the bottom (Fig. 9.7). Using a 50 ml plastic syringe, 35 ml of air is withdrawn from the cylinder through an injection port and immediately replaced with 35 ml of  $C_2H_2$ . The amount of  $C_2H_2$  introduced is equivalent to 10% (v/v) of  $C_2H_2$  in air inside the cylinder. With dense algal mats,  $C_2H_2$  concentration should be doubled. The gas phase is mixed with a 50 ml syringe. To minimize the greenhouse effect, samples are incubated 1 h under 20-30 klx in a light chamber at a constant temperature of 30 °C. Gas samples (0.5 ml) are collected with 1 ml plastic syringes before and after incubation. Ethylene and  $C_2H_2$  in the gas samples are analyzed using a gas chromatograph with a Porapak N column and a  $H_2$  flame ionization detector. Oven temperature of the gas chromatograph is maintained at 60 °C. ARA is expressed in pmole  $C_2H_2$  produced/ $m^2$  per h.

*Selected references on ARA measurement in ricefields:* Eskew 1987, Hardy et al 1973, Kulasooriya et al 1988, Watanabe and Cholitulkul 1979, Watanabe and Roger 1985b, Witty 1983.

### 9.3. Sampling methods for soil and water fauna

Records of the ricefield microfauna and mesofauna usually include zooplankton, molluscs, and soil invertebrates, which are most often dominated by aquatic oligochaetes. We present hereafter the methods of sampling adopted by Simpson et al (1993a,1994b,c) for those three groups. Indeed many variations of these methods exist or can be designed.

#### 9.3.1. Estimation of zooplankton abundance

The method of estimating zooplankton abundance follows that set forth by Simpson et al (1994b). Plastic cylinders (70 mm diam) are inserted into the plot (5 cores are used for a 16-m<sup>2</sup> plot). To prevent organisms from escaping, cores are dropped into position from the bund and pushed into the soil until 2-3 cm remain above water. The enclosed floodwater is sucked into a collection bottle using a vacuum pump. As the water is withdrawn, the cylinder is tilted so that remaining water can be collected in a peripheral depression. Water collected from the cores is combined and the total volume recorded. Samples are poured through a 105- $\mu$ m mesh sieve. Retained material is washed and backwashed into a mesh funnel (128  $\mu$ m) to reduce sample volume. Samples are preserved with 4% formaldehyde and stored. Preserved samples are washed through a series of graded meshes (1000,750,500,350 and 250  $\mu$ m), contained in a plastic funnel with an escape mesh of 128  $\mu$ m. Material retained on each mesh and the escape mesh is backwashed into separate Petri dishes and organisms are counted. Invertebrate groups usually counted are ostracods, copepods, cladocerans, mosquito larvae, chironomid larvae, aquatic insects, and miscellaneous organisms. If no attempt is made to quantify individual species, it is recommended to at least identify the dominant species.

#### 9.3.2. Estimation of the abundance of benthic molluscs

The method used for the enumeration of benthic molluscs (Simpson et al 1994c) is adapted from a procedure described by Grant et al (1985). At each sampling point (3 points are used for a 16-m<sup>2</sup> plot), a 25-  $\times$  25- cm metallic quadrant is inserted between the rice hills. The 2-3 cm of top surface soil are carefully removed by hand and placed into a plastic bag. Replicate samples from each plot are combined for processing. Soil collected is washed through stacked sieves (1- and 2-mm mesh) with a water jet. Material retained on each sieve is backwashed into sorting trays, from where the molluscs are collected by hand. The total number and wet weight of snails retained on each sieve are recorded. Specimens are preserved in 70% alcohol, stored, and identified. The shell lengths of all individuals are recorded.

#### 9.3.3. Estimation of the abundance of aquatic oligochaetes

The method for estimating abundance of oligochaetes follows that set forth by Simpson et al (1993a). Soil for tubificid populations is sampled with plas-

tic tubes (2.7 cm diam, 25 cm long) down to the plow depth. Two sets of 5 cores evenly spaced along a transect are collected from a 16-m<sup>2</sup> plot. Cores are deposited into plastic bags. They are processed in batches of 5, except at the first sampling when all cores are processed separately to permit relations between mean and variance to be established.

In the laboratory, a small quantity of water is added to the sample bags. Contents are gently squeezed to break up any core structure. That reduces 1) the amount of washing required to extract the aquatic oligochaetes, 2) the time required to process each sample, and 3) the possibility of damage to the organisms.

Samples are transferred into a series of graded sieves: mesh sizes 2, 1, and 0.25 mm. The finest mesh used is suitable for the retention of small Tubificidae and Naididae. Soil retained on the mesh is carefully washed away using a fine jet of water. Washing is continued until the percolating water is clear. Depending on the quantity, material retained on each mesh is backwashed into one or more plastic sorting trays.

The aquatic oligochaetes are located by eye, removed with either a fine pair of forceps or a Pasteur pipette, and counted. Samples are sorted live, rather than preserved, because movement greatly increases detectability. Specimens obtained are anesthetized in 10% ethanol, fixed in 4% formaldehyde solution for 24 h, and transferred to 80% ethanol for storage.

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# Keyword index

## A

### Algae

- detrimental effects **45, 46, 52-55, 103,105**
- diatoms **28, 37, 38, 55, 82, 83, 112, 190**
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